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Insecticide Content of Diet and Body Fat of Alaskan Natives

Abstract. A study was made of the DDT and DDE content of the diet and body fat of native Alaskans who lived in isolated, primitive areas and had minimal contact with insecticides. No DDT or DDE was detected in any of the native Alaskan foods analyzed with the exception of two white owls, both of which contained low levels of DDE. Eskimos store considerably less DDT and DDE in their body fat than the general population in the United States. These low dietary levels and the resultant low levels in body fat are consistent with previously published data on the relationship between intake and storage of DDT.

Analysis of representative restaurant (*1*) and institutional (*2*) meals has indicated that the average person in the United States consumes 184 to 202 μg of DDT and even smaller amounts of DDE in his daily food. Most of the material is found in animal fats, and only small amounts in vegetables and other constituents of meals. The total dietary intake accounts for most if not all of the DDT and its metabolite DDE stored in people without occupational exposure to the insecticide.

Hayes and his co-workers (*3*) reported that analysis of fat from persons who died before 1942, and, therefore, before the use of DDT, revealed no trace of DDT-like material. By contrast, samples of body fat collected from the general population during 1954–56 contained DDT in an average concentration of 4.9 parts per million (ppm). The same authors found that meatless meals served in a cafeteria catering to meat abstainers contained only about one-fourth as much DDT as meals served in ordinary restaurants. Persons abstaining from meat deposited in their fat only about half as much DDT as people with an ordinary diet. Thus, for that study, the storage of the insecticide was not only proportional to dietary dosage but also proportional to the intake of meat. Although dosage was probably the important variable, the data offered no way of evaluating any contribution that animal fat may make to the absorption and eventual storage of DDT present in the food.

It is, of course, clear that occupational exposure to DDT may lead to storage far greater than that ever reported as the result of ordinary dietary intake. An average concentration of 17.1 ppm was found in the fat of agricultural workers who applied DDT (*3*). A concentration as high as 648 ppm was found in the fat of an asymptomatic worker in a formulating plant (*2*).

Further search has been made for groups of people with minimal occupational, environmental, and dietary contact with DDT. Native Alaskans who live in an isolated, primitive area where there is little or no use of insecticides and who eat food of local origin appeared to be a group that might have minimal DDT exposure and at the same time maximal intake of animal fat. The present paper describes a study of DDT and DDE content of the diet and body fat from these Alaskan natives.

The food samples were collected in the villages of Shungnak, Kotzebue, Gambell, Hooper Bay, and Point Hope. A total of 42 samples of food, representing 31 different items of the Eskimo diet, were analyzed. The foods studied included various fresh and dried fish; fat, oil, or meat from beaver, beluga, caribou, eider duck, moose, oogruk, polar bear, seal, walrus, whale, and white owl; and miscellaneous foods including cranberries, salmonberries, and wild rhubarb. These foods make up the major portion of the diet of the village Eskimos. There are also a limited number of imported food items, including cereals, sugar, bacon, lard, and hydrogenated fat, in the diet of these people.

All analyses for DDT and DDE in both food and body fat were carried out by the modifications of the Schechter-Haller spectrophotometric procedure cited by Hayes *et al.* (*3*).

No DDT or DDE was detected in any of the native foods analyzed, with the exception of two white owls from Point Hope, which contained 1.1 ppm DDE in the meat. The source of exposure of these birds to DDT is unknown. It may be accounted for by their migratory habits. It is also possible that some naturally occurring constituent of food stored by these birds may interfere in this analysis.

Samples of human body fat were obtained through the cooperation of the U.S. Public Health Service Hospital in Anchorage. Single samples from 20 patients were analyzed. The subjects chosen were residents of isolated villages. Fat samples were taken from patients who underwent surgery after a minimal period of hospitalization. Sample meals from this hospital for one day were analyzed and found to contain 184 μg of DDT and 26 μg of DDE. A dish of meatballs and spaghetti contained 111 μg of DDT and represented 60 percent of the daily total residue of this insecticide in the diet. These daily totals for DDT and DDE are similar to those found in representative restaurant and institutional meals in the 48 contiguous states (*1, 2*).

The DDT and DDE content of the body fat of these native Alaskans is shown in Table 1, along with comparative values obtained for the general population and for meat abstainers by

Table 1. Storage of DDT and DDE in the fat of Alaskan natives (as found in this study) in comparison with the general population of the United States and with abstainers from meat (as found by Hayes *et al.*, *3*).

Value	DDT (ppm)		DDE (ppm)	
	Tissue	Extract*	Tissue	Extract*
Alaskan natives (20 cases)				
Range	0 to 1.9	0.3 to 2.2	0 to 3.9	2.5 to 5.8
Mean ± S.E.	0.8 ± 0.10	1.4 ± 0.16†	2.0 ± 0.41	3.8 ± 0.31†
General population (61 cases)				
Range	2 to 12	3 to 22	2 to 13	3 to 25
Mean ± S.E.	4.9 ± 0.35	6.8 ± 0.42	6.1 ± 0.42	8.6 ± 0.52
Abstainers from meat (16 cases)				
Range	0 to 7	0 to 10	0 to 9	0 to 12
Mean ± S.E.	2.3 ± 0.44	3.5 ± 0.63	3.2 ± 0.63	4.9 ± 0.84

* Carbon tetrachloride extract. † Based on 11 samples only.

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Hayes *et al.* (*3*). It is apparent that these Eskimos stored considerably less DDT and DDE than persons in the general population of the United States and somewhat less than meat abstainers. The DDE constituted from 10 to 90 percent and averaged about 73 percent of the total DDT-derived material for the Eskimo. For the general population this percentage was 56 and for meat abstainers, 59. These figures support a view that individuals with a lower degree of exposure to DDT are able to convert DDT to DDE more efficiently. The low storage level is consistent with the low level of the insecticide and its metabolite found in the Eskimo diet in spite of the very high meat content of that diet. The small amount of DDT and DDE that was found in the fat of the native Alaskans is probably accounted for by their limited consumption of imported foods and by their brief intake of hospital food prior to surgery.

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Toxicity of pesticides to man: risks from present levels

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INTRODUCTION

As background for the evaluation of safety undertaken in this paper, I propose to review: (*a*) evidence that all modalities of toxicity exhibit a dosage-response relationship, and (*b*) the quantitative importance of factors which are known to influence toxicity. It is only through an appreciation of the critical importance of the dosage-response relationship, and thorough knowledge of the limited degree of difference which may be produced by various factors when the dosage is constant, that we can have assurance of the safety of any pesticide, drug, food, or other chemical when used under specified conditions.

Control of dosage is the basis of all safety in the use of chemicals. This rule applies not only to compounds of relatively high toxicity but also to compounds of low toxicity including those necessary to life. Babies have been killed by putting salt in their formula in place of sugar (Finberg *et al.* 1963), and it is said that the ancient Chinese carried out executions using water as a toxicant. On the other hand, all of us tolerate traces of arsenic, lead, and mercury (Monier-Williams 1949) which are naturally occurring elements widely distributed in food and water. They are found in marine fish and in undeveloped areas where they have no use in industry or as pesticides.

DOSAGE-RESPONSE RELATIONSHIPS

Acute and chronic toxicity

All people with toxicological or medical training are aware that toxicity in the restricted sense corresponds to dosage for any particular compound. In the case of acute toxicity, the relationship between dosage and effect is easily expressed by *LD*50 and related statistics. Although expression of the effects of repeated or continuous dosage is more complex, there can be no doubt that they are dependent on dosage. For a number of reasons, emphasis is placed on finding the highest dosage of a compound which produces no detectable or injurious effects even when administered for the lifetime of the individual.

The less common or less familiar a phenomenon is, the more likely that its relationship to an actual or supposed aetiology will be viewed qualitatively rather than quantitatively. Phenomena often viewed in this way include storage, potentiation, neurotoxicity, teratogenesis, and carcinogenesis as well as the adaptive response of microsomal enzymes.

Metabolism and storage

People trained in the biological sciences are generally familiar with the fact that compounds which are absorbed tend to reach a steady state of storage and that for each compound the storage at equilibrium corresponds to dosage. People not trained in the biological sciences are usually unfamiliar with these facts and suppose that compounds are of two distinct sorts, those that are stored and those that are not. They suppose that compounds that are stored at all continue to accumulate indefinitely with no tendency to reach a steady state in which the amount lost each day is equal to the amount absorbed. It may seem odd to mention this folklore in a conference such as this, but the views of the public are a most important factor, which must be taken into account in any long-term effort to achieve general public acceptance of a particular usage or course of action.

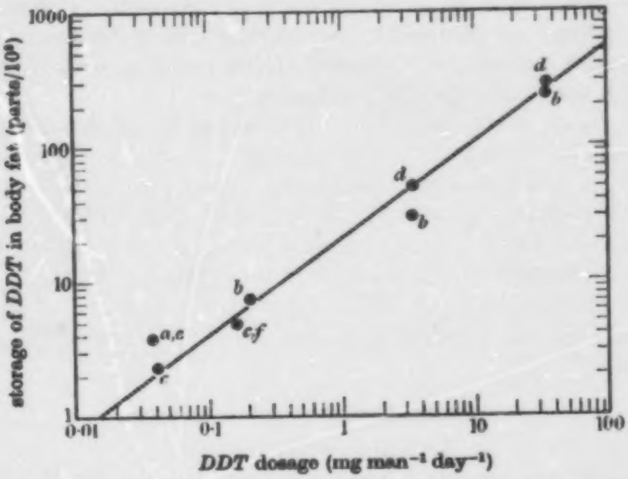

FIGURE 1. Storage of *DDT* in the body fat of people in relation to dosage. Reprinted from Durham *et al.* (1965) by permission of the authors and the American Medical Association. The original sources of the data are indicated by letters as follows: *a*, Durham *et al.* (1965); *b*, Hayes *et al.* (1956); *c*, Hayes *et al.* (1958); *d*, Hayes *et al.* (in preparation); *e*, Quinby *et al.* (1965); *f*, Walker *et al.* (1954).

The relation between equilibrium-storage and dosage for *DDT* in man is shown in figure 1 taken from a recent publication by Durham *et al.* (1965). We will return to this figure later in order to estimate the absorption of *DDT* in different countries and relate this dosage to levels that are considered safe.

Potentiation

True potentiation is a comparatively rare phenomenon except in connexion with the organic phosphorus insecticides. When potentiation does occur, it almost always involves one or a few relatively large doses rather than doses small enough that they may be tolerated for an extended period. The reason is that much potentiation is best explained by competition of two or more compounds for a common enzyme site. This is the mechanism that explains the potentiation of the toxicity of malathion by *EPN* when the dosage of both compounds is substantial (Murphy & DuBois 1957). However, if the dosage of the two compounds is sufficiently small, there is enough enzyme to detoxify both of them, and the phenomenon of potentiation is not manifest. Rider *et al.* (1959) have shown that people can tolerate 3 mg/day of *EPN* plus 16 mg/day of malathion or 6 mg/day of *EPN* plus 8 mg/day of malathion for prolonged periods without significant depression of red cell or plasma cholinesterase. The combination of 6 mg/day of *EPN* plus 16 mg/day of malathion did produce asymptomatic depression of both enzymes, but the effect was only additive. No potentiation was noted. Thus, potentiation among this class of compounds may be important for overexposed workers but not for people who eat ordinary residues.

It seems likely that the mechanism of cocarcinogenesis will not be explained until neoplasia itself is explained. However, in what may have been the only quantitative study of this kind of potentiation, a dosage–response relationship was found not only for 9,10-dimethyl-1,2-benzanthracene but also for it in combination with the carcinogen croton oil (Graffi 1953).

Neurotoxicity

By neurotoxicity is meant the delayed but persistent paralysis caused by certain organic phosphorus compounds. The classical example is 'jake leg' paralysis caused by triorthocresyl phosphate. Most studies of neurotoxicity have attempted to learn which molecular configurations are capable of producing the phenomenon and which are not. When active compounds were investigated quantitatively, it was found that a sufficiently low dosage was tolerated, and progressively larger dosages increased the frequency and severity and often reduced the latency of neurotoxicity (Davies *et al.* 1960; Aldridge & Barnes 1961; Cavanagh *et al.* 1961; Siegel *et al.* 1965).

Teratogenesis

Although most research in teratogenesis has been centred on the nature of the phenomenon itself and the biological factors which influence it, a few quantitative studies have been made, and they illustrate that for any given compound and experimental situation there is a dependable relationship between dosage and effect (Murphy 1965). It has been pointed out that there is no way to exclude that any given compound may be teratogenic to some species under certain conditions (Bough *et al.* 1963; Karnofsky 1965).

Carcinogenesis

Figures 2 and 3 show that recognized strong carcinogens show a striking dosage-response relationship. However, so much research on carcinogenesis has been centred on the phenomenon itself and so little attention has been given to quantitation of the actions of chemicals that even some experts in the field seem unfamiliar with the fact that chemical carcinogenesis follows clearcut dosage-response relationships.

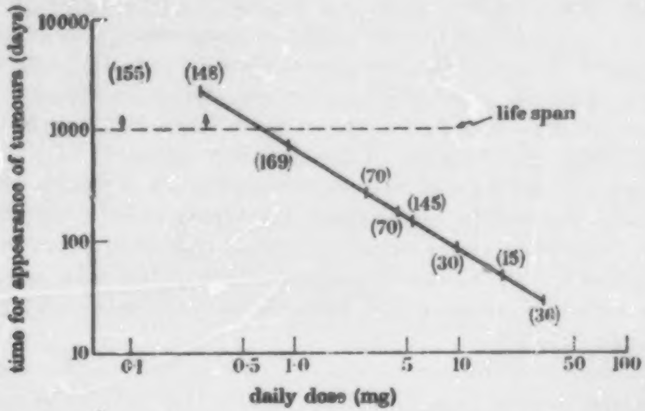

Figure 2. Response of rats to graded daily doses of *p*-dimethylaminoazobenzene administered orally. No liver tumours were obtained with the two lower doses employed. Number in parentheses refers to numbers of animals at each level. The appearance of the first tumour was considered the end point for each dosage group. Reprinted with permission from the National Academy of Sciences—National Research Council (1960).

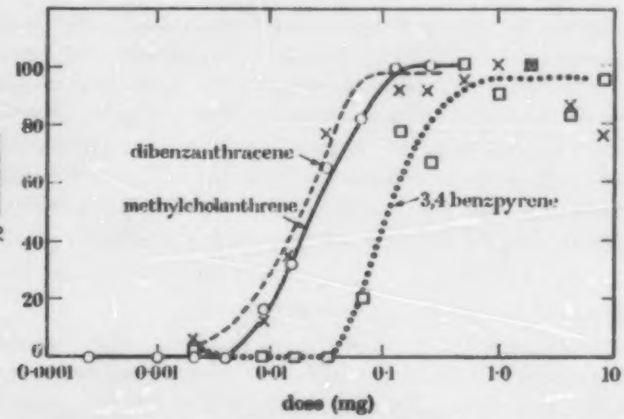

Figure 3. Response of mice to graded single doses of each of three polycyclic aromatic hydrocarbon carcinogens dissolved in tricaprylin and injected subcutaneously. Approximately twenty animals per dose of each compound. Reprinted with permission from the National Academy of Sciences—National Research Council (1960).

Induction of microsomal enzymes

One of the most exciting developments in recent years concerns the microsomal enzymes. They help to explain a number of otherwise obscure facts in pharmacology. It is generally admitted that their net effect is adaptive, although some persons fear that stimulation of enzymes by one chemical will lead to greater injury to the organism when faced with some other challenge. Biologists tend to believe firmly in adaptation, but they are often sceptical about specific examples. Regardless of the final toxicological evaluation of the process of induction, it is clear from work already complete that there are orderly relationships between dosage and response for compounds which stimulate microsomal enzymes (Kinoshita *et al.* 1966; Durham to be published). Kinoshita *et al.* (1966) have made great progress by giving as much attention to the different dosages of stimulator as has generally been given to measuring the degree of response of the enzymes to stimulation. More work of this kind is needed.

Hypersensitivity and allergy

The fact that some people are 'allergic' and others are not tends to obscure the fact that the various forms of hypersensitivity are dose-related within a homogeneous population. Individuals who suffer from allergy often find that a reduction of dosage will lead to clinical improvement. For example, people who are sensitive to pollen often get some relief by closing most of the air inlets of their homes and placing filters on the remaining ones, even though this procedure does not eliminate their exposure to pollen but merely reduces it. Under experimental conditions in which the susceptibility of animals was made uniform by passive transfer, the onset of anaphylaxis was directly related to the dose of antigen (Pruzansky *et al.* 1959). Human leukocytes isolated from ragweed-sensitive donors release histamine at rates determined by the concentration of purified antigen derived from the pollen (Lichtenstein & Osler 1964). Other forms of hypersensitivity may be dose-related also. For example, blood dyscrasias, especially aplastic anaemia, are a recognized hazard of the otherwise valuable drug chloramphenicol. Hodgkinson (1954) showed that these dangerous side effects occurred predominantly in cases in which the drug had been administered at a rate significantly higher than usual.

The fact that even hypersensitivity often is dose-related emphasizes the importance of searching for suspected but undemonstrated dangers of a particular compound among people whose exposure is most intensive and prolonged.

FACTORS OTHER THAN DOSAGE THAT INFLUENCE TOXICITY

Although dosage is the main factor determining whether a particular chemical will or will not produce a given effect, there are other factors that influence response. Factors of a biological nature include route of exposure, species and individual differences, sex, age, nutrition, and disease. Physicochemical factors include temperature and other environmental variables and, of course, the schedule and duration of dosage and the formulation in which the chemical is administered. There is no theoretical and frequently little practical limit to the range of dosage which may be explored experimentally, and there is frequently little practical limit to the range of human dosage that may be encountered at least occasionally. Thus, at one end of the spectrum it may be possible to find unexposed populations and at the other end of the spectrum to find an occasional person who is killed by accidental ingestion of a single large dose. Compared to dosage, the other factors that influence response to a particular chemical have been subject to less quantitative study. However, it is important that we learn as much as possible about the magnitude of the variation from these other sources in order properly to evaluate safety.

Schedule of dosage

It is common knowledge among toxicologists that the schedule of dosage may have an important influence on the quantitative results. Usually anything that permits greater detoxification or excretion of a toxin tends to reduce the injury it produces. Thus, ingestion of a certain daily dosage mixed in the diet often is less injurious than the same dosage of the same compound administered daily by stomach tube. The compound reaches a lower maximal concentration in blood and other tissues when the same dosage is distributed throughout the day rather than being concentrated in a brief period. The microsomal enzymes, excretion, and other defences may be able to cope indefinitely with a low concentration of compound but may not be capable of handling peak levels.

Similar reasoning applies to schedules that permit rest periods as compared to those that do not. Truly continuous exposure is usually more damaging than intermittent exposure at the same daily rate. An example may be cited for lead (Kehoe 1961).

The general rule that rest periods and avoidance of peak blood levels tend to be protective usually applies best to compounds that are easily detoxified and excreted and least to compounds against which the defences of the body are inherently poor with the result that the compounds or their effects are relatively cumulative. The cumulation may occur over relatively long periods as, for example, with lead or over short periods as, for example, with carbon monoxide. In the former case, the cumulation frequently involves months. In the latter case, the cumulative effect may involve hours or days but is not prominent in connexion with longer periods. In fact, for carbon monoxide and some other toxic gases, the degree of injury (over a certain range of concentrations and durations) is similar for all situations in which the product of concentration of gas times duration of exposure is a constant. This relationship is spoken of as Haber's law. However, at sufficiently low concentrations the 'law' does not operate. All of us inhale some carbon monoxide, and those who smoke tobacco inhale more than non-smokers (Hanson & Hastings 1933). Some garage workers encounter a level of exposure that is marginal in respect to injury. Higher levels of exposure involve progressively more hazard with the result that in some countries carbon monoxide kills more people than any other single compound.

In a few instances (Saffiotti & Shubik 1956; Waud *et al.* 1958), repeated small doses produce greater injury than a single dose even though the total dosage resulting from the repeated application is less than that from the single one. This relationship would appear to violate a dose–response relationship. The explanation may involve a failure of the single dose to reach the target tissue. Prolonged action may be required when there is an inherent delay between the initial dosage and first observed effect. However, a delay in onset of injury may follow a single dose as with the neurotoxic effects of some organic phosphorus compounds, the anticoagulant effects of some coumarin derivatives, or the action of some carcinogens (figure 3). The mechanism of delay appears to be best understood in connexion with the anticoagulant rodenticides, which interfere promptly with the formation of prothrombin but have no effect on the rate of disappearance that determines the delay.

The modifying effect of schedule and a number of other factors must be taken into account in any consideration of dosage. Tests to establish safe levels should involve the dosage schedule, route, and other conditions people are expected to encounter. When these modifying factors are taken into account, the paramount importance of dosage becomes even more evident.

Duration of dosage

Weil & McCollister (1963) investigated the degree of toxicity revealed by short-term and long-term tests in rats. For twenty-two compounds, the ratios of the dosages producing the minimal effect observed in short-term and in 2-year feeding tests varied from 0·5 to 20·0 and averaged 2·9. Ratios greater than 1·0 indicated the degree of apparent increase in toxicity associated with long-term testing. Ratios less than 1·0 may have indicated adaptation, experimental variation, or both. It was possible to compare the maximal dosages of 33 compounds producing no effect; these ratios ranged from 0·5 to 12·0 and averaged 2·3.

Route of exposure

The route by which a compound is absorbed helps to determine not only the ease of absorption, but also, in some instances, the ease of metabolism. Compounds are usually more toxic by the oral than by the dermal route, and this was true of 64 of the 67 compounds studied in this regard by Gaines (1960; also in preparation). However, there were three exceptions, that is three compounds more toxic by the dermal route. Considering all 67 compounds, the factor of difference by which oral toxicity exceeded dermal toxicity ranged from 0·2 to 21 and averaged 4·2. The lesser toxicity of one of the compounds (Isolan) by the oral route was markedly influenced by metabolism. Five of six rats survived infusion of Isolan into an intestinal vein for an hour at a rate that led to death within 18 to 35 min in six comparable animals infused via the femoral vein (Gaines *et al.* 1966). Thus, a single pass through the liver is sufficient to make the difference between life and death in connexion with Isolan, and this phenomenon helps to explain the high dermal, low oral toxicity of the compound.

For practical reasons, respiratory toxicity is studied and reported in terms of concentration of chemical and duration of exposure. Values obtained in this way cannot be converted easily to dosage in terms of body weight, and direct comparison with toxicity by other routes is not usually possible. Some notion of the respiratory toxicity of a compound may be inferred from its intravenous toxicity (DuBois & Geiling 1959). However, the method is of limited value partly because gases and aerosols are absorbed by the respiratory tract to different degrees depending on the compound and the particle size.

Species and strain differences

Variation between species must be considered every time a new compound which has been properly tested in animals is used for the first time. It is frequently suggested that the tests be made in a large number of species. If the results in the different animals are similar, it is likely that human response to the compound will not be greatly different. If, on the contrary, there is a wide variation in the response of different species, then conservatism forces us to suppose, until there is direct evidence to the contrary, that man may be at least as sensitive as the most susceptible species. Apparently norbormide shows the widest range of susceptibility between species. The *LD* 50 for albino Norway rats is 4·3 mg/kg, while dogs, cats, monkeys, sheep, chickens and turkeys are unaffected by single doses of 1000 mg/kg (Roszkowski *et al.* 1964; Roszkowski 1965). Thus, the factor of difference for these species for norbormide is greater than 230. Although this is an unusual situation, it emphasizes how different from others one species can be. Even the wild strain of Norway rat is less susceptible (*LD* 50, 12 mg/kg). Examination of table 7 shows that for many pesticides the factor of difference in susceptibility of the mouse, guinea-pig, rabbit, and dog ranges from 0·2 to 11·8 and averages

TABLE 7. RELATIVE SUSCEPTIBILITY OF DIFFERENT SPECIES TO PESTICIDES BASED ON ORAL *LD*50 VALUES (GAINES 1960; FAO–WHO 1965*b*; LEHMAN 1965) AND USING THE MALE RAT AS STANDARD

	susceptibility factor*			
compound	mouse	guinea-pig	rabbit	dog
chlorobenzilate	1·4	—	—	—
DDT	0·3–0·8	0·3	0·3–0·5	—
methoxychlor†	2·7–3·8	—	—	—
lindane	1·0	0·7–0·9	0·4–1·5	—
aldrin	0·9–1·2	1·2–1·6	0·5–1·1	0·4–0·8
chlordane	0·8	—	1·1–3·4	—
dieldrin	1·2	0·9–1·0	0·9–1·0	0·7–0·8
endrin	—	0·5–2·7‡	1·8–6·2	—
heptachlor	0·6–1·5	0·8–0·9	—	—
azinphosmethyl	1·6	0·2	—	—
Chlorthion	0·7	—	—	—
diazinon	0·9–1·4	0·3	0·8	—
dimethoate	3·0–5·4	0·5–0·9	—	—
dioxathion	—	—	—	1·1–11·8
malathion	1·6–1·9	—	—	—
methyl parathion	0·4	—	—	—
mevinphos	0·9–1·6	—	—	—
oxydemetonmethyl	1·0–2·5	0·3–0·6	—	—
parathion	0·2–5·0	1·6–3·2	0·5–3·0	—
phosphamidon	1·8	—	—	—

* A factor of less than 1·0 indicates less susceptibility than that of the male rat; a factor greater than 1·0 indicates greater susceptibility.

† Both sexes.

‡ Approximate.

close to 1·0 as compared to the susceptibility of the male rat. The fact that the species difference is usually small is confirmed by comparisons based on tests which may be applied to people as well as to experimental animals or on information obtained in connexion with accidents to man. Such comparisons of the susceptibility of man and animals are shown in tables 8 and 9. Inspection of the tables

TABLE 8. COMPARISON OF THE SUSCEPTIBILITY OF MAN AND OTHER ANIMALS TO CERTAIN PESTICIDES. ALL DOSES ARE SINGLE AND ORAL UNLESS OTHERWISE NOTED

		dosage (mg/kg)								
compound	species	largest without clinical effect	smallest with clinical effect	median clinical *CD* 50	smallest with serious effect	largest nonfatal	smallest fatal	*LD* 50	uniformly fatal	references
DDT	Man	—	6	10	16*	285†	—	—	—	Garrett 1947; Hsieh 1954; Neal *et al.* 1946; Velbinger 1947; Hayes 1959
	rat, F	—	—	—	75	150	100	118	200	Gaines‡
	rat, M	25	—	—	50	175	50	113	200	Gaines‡
lindane	man	—	0·4§	0·4§	0·5*	—	—	—	—	Graeve and Herrnring 1951
	rat, F	—	—	—	—	125	75	91	—	Gaines‡
	rat, M	—	—	—	50	125	75	87	200	Gaines‡
	calf	2·5	—	—	5	—	—	—	—	Radeleff *et al.* 1955
chlordane	man	—	—	—	32*†	—	—	—	—	Dadey & Kammer 1953
	man	—	—	—	—	—	29–57	—	—	Hayes 1963
	infant	—	—	—	10*	—	—	—	—	Lensky & Evans 1952
	rat, F	100	200	—	300	550	350	430	600	Gaines‡
	rat, M	—	—	—	250	400	250	335	450	Gaines‡
dieldrin	man	—	—	—	10	—	—	—	—	Princi 1952
	rat, M, F	—	—	—	30	60	30	46	—	Gaines‡
endrin	man	—	—	—	0·2	—	—	—	—	Davies & Lewis 1956
	rat, F	—	—	—	6	10	6	7·5	—	Gaines‡
	rat, M	—	—	—	10	25	10	17·8	30	Gaines‡
DDVP	man	—	—	—	51	—	—	—	—	Hayes 1963
	rat, F	—	—	—	—	100	37	56	125	Gaines‡
	rat, M	—	—	—	—	125	75	80	150	Gaines‡
	cow	—	—	—	27	—	—	—	—	Tracy *et al.* 1960
	horse	—	—	—	25	—	—	—	—	Jackson *et al.* 1960
diazinon	man	—	—	—	2·2‖	—	—	—	—	Hayes 1963
	man	—	—	—	—	250	—	—	—	Bockel 1957
	rat, M	—	—	—	200	300	200	250	350	Gaines‡
	calf	—	—	—	1	—	10	—	—	Radeleff *et al.* 1955
malathion	man	—	—	—	—	200	71	—	—	Walters 1957; Paul 1960
	rat, F	—	—	—	750	1250	750	1000	1500	Gaines‡
	rat, M	500	—	—	1000	1750	1000	1375	2000	Gaines‡
	sheep	150	100	—	100	300	150	—	—	Radeleff *et al.* 1955
parathion	man	—	—	—	—	6·4	2·0	—	13	Goldblatt 1950; Hayes 1963
	child	—	—	—	—	—	0·1	—	—	Kanagaratnam *et al.* 1960
	rat, F	—	—	—	1	4·5	3·0	3·6	5	Gaines‡
	rat, M	5·0	—	—	10	20	10	13·0	30	Gaines‡
	calf	—	—	—	0·5	—	1·5	—	—	Radeleff *et al.* 1955
	sheep	50	—	—	—	75	20	—	—	Radeleff *et al.* 1955
	steer	25	—	—	—	—	—	—	—	Radeleff *et al.* 1955
TEPP	man	—	0·05	—	3·5	—	—	—	—	Grob & Harvey 1949; Grob *et al.* 1950
	rat, M	—	—	—	—	—	1·0	1·05	—	Gaines‡

* Convulsions; † part of dose vomited; ‡ based partly on published papers (Gaines 1960; also in preparation) and partly on the original data from which the papers were drawn; § 3 times a day for 3 days; highly dispersed formulation; ‖ dermal.

Table 9. Comparison of the susceptibility of man and other animals to repeated doses of certain pesticides. All doses are oral unless otherwise noted

compound	species	dosage (mg kg⁻¹ day⁻¹)	duration (days)	results	references
DDT	man	0·5	> 600	increased storage; no clinical effect	Hayes *et al.* 1956
	rat, M, F	0·24	161	histopathological changes of the liver	Laug *et al.* 1950
methoxychlor	man	2	56	no effect	Stein *et al.* 1965
	rat, M, F	4·37	750	no effect level	Lehman 1965
demeton	man	0·05	24	15% reduction of plasma ChE only	Moeller & Rider 1962
	rat, F	0·05	112	no significant depression of ChE	Barnes & Denz, 1954*b*
	rat, F	0·14	112	30% inhibition of ChE	Barnes & Denz 1954*b*
	rat, F	0·24	66	60% reduction of plasma and 40% reduction of RBC ChE	Gaines, unpublished results
	dog, M, F	0·025	168	no significant depression of ChE	Frawley & Fuyat 1957
	dog, M, F	0·047	168	significant depression of ChE	Frawley & Fuyat 1957
dimefox	man	0·002	70	no effect on ChE	Edson *et al.* 1964
	man	0·0034	70	25% reduction of whole blood ChE	Edson *et al.* 1964
	rat, F	0·024	28	about 50% inhibition of RBC ChE; no effect on plasma ChE	Edson 1956; Edson *et al.* 1964
	rat, F	0·095	28	75% reduction of RBC ChE; 25% reduction of plasma ChE	Edson 1956; Edson *et al.* 1964
	rat, F	0·475	28	almost complete inhibition of RBC ChE; 75% reduction of plasma ChE	Edson 1956; Edson *et al.* 1964
dioxathion	man	0·05	59	no inhibition of RBC and plasma ChE	Frawley *et al.* 1963
	man	0·1	28	slight inhibition of plasma ChE; no effect on RBC ChE	Frawley *et al.* 1963
	rat, M, F	0·22	91	no significant effect on ChE	Frawley *et al.* 1963
	rat, M, F	0·78	91	significant reduction of RBC & plasma ChE	Frawley *et al.* 1963
	dog, M, F	0·25	12	slight inhibition of plasma ChE; no effect on RBC ChE	Frawley *et al.* 1963
	dog, M, F	0·8	12	marked effect on plasma ChE; no effect on RBC ChE	Frawley *et al.* 1963
malathion	man	0·34	56	maximal reduction of 25% plasma & RBC ChE	Moeller & Rider 1962
	rat, F	3·2	90	29% reduction in RBC and no reduction of plasma ChE on 30th day; recovery by 90th day	Gaines, unpublished results
	rat, M	4·5	730	10 to 30% inhibition of plasma and RBC ChE	Hazleton & Holland 1953

TABLE 9 (*cont.*)

compound	species	dosage (mg kg^{-1} day^{-1})	duration (days)	results	references
methyl parathion	man	0·1	24	15% reduction of plasma ChE only	Moeller & Rider 1962
	dog, M, F	0·94	84	significant depression of plasma and RBC ChE	Williams *et al.* 1959
parathion	man	0·1	42	33% reduction of whole blood ChE; 16% inhibition of RBC ChE; 37% inhibition of plasma ChE	Edson *et al.* 1964
	rat, F	0·07	90	no effect	Gaines unpublished results
	rat, F	0·26	84	80% reduction of RBC ChE; slight inhibition of plasma ChE	Edson *et al.* 1964
	rat, F	0·35	90	37% reduction of plasma and 44% reduction of RBC ChE	Gaines unpublished results
	dog	0·047	168	60% inhibiton of plasma ChE	Frawley and Fuyat 1957
	pig	4·0	49	80% inhibition of RBC ChE; no inhibition of plasma ChE	Edson *et al.* 1964
schradan	man	0·014	44	25% reduction of blood ChE	Edson *et al.* 1964
	man	0·06	60	77% inhibition of RBC ChE; 50% inhibition of plasma ChE	Edson *et al.* 1964
	rat, M, F	0·045	112	substantial reduction of RBC ChE; no effect on plasma ChE	Edson *et al.* 1964
	rat, M	0·22	14–85	complete inhibition of RBC ChE	Edson *et al.* 1964
	pig, F	0·1	102	55% inhibition of RBC ChE; slight reduction of plasma ChE	Edson *et al.* 1964
arsenic trioxide	man	0·44	?	frequent mild poisoning	Sollmann 1957
	sheep	10	—	tolerated without symptoms	Reeves 1925
	horse	4·7	—	tolerated without symptoms	Reeves 1925
warfarin	man	0·14	indef.	maintenance therapeutic dose	Friedman 1959
	man	0·29–1·45	15	haemorrhage in 12 people (4 to 70 y) followed by recovery	Lange & Terveer 1954
	man	1·7	6	haemorrhage in 22 y man followed by recovery	Holmes & Love 1952
	man	0·83–2·06	15	fatal to boy (19 y) and girl (3 y)	Lange & Terveer 1954
	rat, M, F	0·08	40	killed 5 of 10	Hayes & Gaines 1959
	rat, M, F	0·39	15	killed all of 10 rats	Hayes & Gaines 1959
2,4-D	man	14–37	18	tolerated	Seabury 1963
	man	66	1	coma, hyporeflexia; incontinence	Seabury 1963
	rat, F	15	112	tolerated	Hill & Carlisle 1947
	dog	9	84	tolerated	Drill & Hiratska 1953

indicates, for example, that man is more susceptible than the rat to lindane, about equally susceptible as the female rat to parathion, and distinctly less susceptible to warfarin than rats of either sex. Although in many instances in which a direct comparison is possible there is no marked difference, the difference appears to be about 100-fold for a few compounds. Furthermore, the tables by necessity involve phenomena that can be studied in laboratory animals. Much of the reason for making tests in man is the existence of hypersensitivity, subjective responses, and other phenomena that do not lend themselves to study in animals.

Individual differences

Individual differences are apparent in every toxicological test, including those carried out on people. Study of a recent paper by Gaines (in preparation), in which he reports the acute oral toxicity of 69 pesticides, shows that the *LD*50 value for male rats ranged from 1·20 to 7·14 times greater than the corresponding *LD*1 value. The average factor of difference was 2·42. The corresponding factors of difference for the dermal toxicity of 42 pesticides were 1·37 to 14·93 with an average of 3·00. In other words, judged in this way individual variation although very real is usually relatively small. In studies of storage and excretion, the greatest individual average excretion of malathion-derived material differed from the smallest individual average excretion at the same dosage by factors of only 2·2 to 8·7 for different groups of people (Hayes *et al.* 1960). Thus, the degree of difference was relatively constant in tests carried out at different dosage levels or at different times. A similar observation was made regarding the storage of *DDT* and the excretion of *DDA* in man. In separate tests, the maximal storage of *DDT* was 1·3 to 5·9 times the minimal storage at the same dosage level. For a single dosage group, the maximal rate of excretion of *DDA* by one man in any one day was 18·0 times greater than his own minimal rate, and the difference between the lowest minimum and highest maximum within the group was 21·5 times (Hayes *et al.* in preparation). In all these tests the relative constancy of one individual as compared with another was noted.

Sex

Chiefly because of its convenience, the rat is used more than any other species for studies in toxicology. The rat also has the apparent distinction of showing more variation between the sexes in its response to chemicals than any other species. This fact may have led to more concern than is justified regarding possible differences in the susceptibility of men and women to chemicals. In any event, calculations from data provided by Gaines (1960; also in preparation) for the oral toxicity of 65 pesticides showed that the difference in the oral *LD*50 for male and female rats ranged from 0·21 (indicating greater susceptibility of the female) to 4·62 (indicating greater susceptibility of the male), and averaged 0·94. The corresponding factors for the dermal toxicity of 37 pesticides were 0·11 to 2·93 with an average of 0·81.

In contrast to the situation in rats, and to a lesser degree other rodents, significant differences between the sexes of other species in their susceptibility to poisons usually have not been reported. Such differences were looked for but not found in relation to the storage of *DDT* in monkeys (Ortega *et al.* 1956; Durham *et al.* 1963). Robinson *et al.* (1965) found somewhat more dieldrin, *p,p′-DDE*, and total *DDT*-derived material in fat from men than in fat from women; in some instances the differences were statistically significant. Zavon *et al.* (1965) observed the same relationship for dieldrin, *DDE*, *p,p′-DDT*, *o,p′-DDT*, and heptachlor epoxide but failed to report the significance. Certain other authors failed to find a difference between men and women in the storage of *DDT* in fat (Laug *et al.* 1951; Hayes *et al.* 1958; Maier-Bode 1960; Read & McKinley 1961; Dale *et al.* 1965; Egan *et al.* 1965; Wassermann *et al.* 1965). However, Dale *et al.* (to be published) found that plasma and whole blood levels of chlorinated hydrocarbon insecticides were significantly higher in men than in women, but the differences did not exceed a factor of 7.

Age

It is now well known that a number of drugs are poorly metabolized by infants, particularly prematures (Fouts & Adamson 1959). Although it is seldom possible to quantitate the difference, it is clear that a dosage of some drugs which is easily tolerated by human adults may lead to severe illness or even death in very young children. Systematic study of drugs (Hoppe *et al.* 1965; Yeary & Benish 1965) and pesticides (Brodeur & DuBois 1963; Lu *et al.* 1965) indicates that newborn animals are generally more susceptible than adults of the same species. However, the difference may be small, and there are some exceptions in which the newborn are actually less susceptible than adults (Brodeur & DuBois 1963; Hoppe *et al.* 1965). As reviewed by Durham (to be published), the difference, no matter what its direction, can generally be explained in terms of the recognized activity of microsomal enzymes in activating or deactivating the chemical in question. Other factors that may be of importance are renal function and membrane permeability. Fortunately, compounds of similar pharmacological action tend to show similar differences in their toxicity to young and adult animals (Yeary & Benish 1965). In studying drugs, Yeary & Benish (1965) found that newborn rats were 0·6 to 10·0 times more susceptible than adults. Hoppe *et al.* (1965) found a similar range of 0·7 to 6·2. In their studies of 15 organic phosphorus insecticides or defoliants, Brodeur & DuBois (1963) found that weanling male rats were 0·2 to 4·1 times more susceptible than adults with a mean of 1·8.

Nutrition

Apparently only extremes of nutrition have produced observable alterations in the toxicity of pesticides. As reviewed elsewhere (Hayes 1959), various mammals and even fish are relatively resistant to poisoning by *DDT* if they are fat rather than thin. The same result has been produced with dieldrin under experimental conditions (Barnes & Heath 1964). Other factors may be involved, but certainly distribution of the insecticide to adipose tissue tends to reduce the concentration of the insecticide at the site of action and thus protects the organism.

If *DDT* is stored in body fat in sufficient concentration, rapid mobilization of the fat through starvation may lead to poisoning (Fitzhugh & Nelson 1947) through increase in the concentration of poison in the small amount of fat which remains and, by the same token, in all tissues of the body (Dale *et al.* 1962). During mobilization of *DDT*, excretion is increased by a factor of about 1·4, but the increase is insufficient to prevent poisoning in some rats. It was pointed out by Dale *et al.* (1962) that starvation is unlikely to precipitate poisoning by *DDT* in man because even people with heavy occupational exposure to the compound do not store enough of it to produce the effect and because the metabolism of man is inherently slower than that of the rat so that man cannot starve so fast.

Some other chlorinated hydrocarbon insecticides are excreted more efficiently than *DDT*. For example, Heath & Vandekar (1964) found that the average excretion of dieldrin in rats was relatively rapid (5%/day), and that it was more than doubled following a few days of starvation. Therefore, it is not astonishing that it was not possible to precipitate dieldrin poisoning in rats by starving them after they had been fed for 7 to 18 months at dietary levels up to 15 parts/10^6 (Treon & Cleveland 1955). A farmer, whose last exposure to dieldrin was about a month earlier, became sick when he took amphetamine sulphate for weight reduction (Paul 1959). The original diagnosis of dieldrin poisoning is open to grave doubt because the symptoms were not characteristic and the man's fat contained only 5 to 7 parts/10^6 of dieldrin (a level sometimes exceeded by healthy workers and exceeded in all undisputed cases of poisoning by dieldrin reported so far).

In addition to the action involving storage just discussed, nutrition may influence toxicity through metabolism promoted by the liver microsomal enzymes. Murphy & DuBois (1958) showed that male rats maintained on a protein-free diet for 4 weeks had only 24% as much microsomal enzyme activity as normal rats. Also, the liver enzymes of rats which did not receive protein could not be induced by compounds which ordinarily stimulate these enzymes.

Disease

Few studies have been made of the relationship between the toxicity of chemicals and the occurrence of disease of other cause. A few exceptions are clearly recognized; for example, silicosis predisposes to tuberculosis. A dosage-response relationship appears to hold, for there is no evidence that inhalation of silica insufficient to cause silicosis has any effect on the occurrence of tuberculosis.

In a study of the interaction of trichinosis and *DDT* poisoning (Hayes in preparation), it was found that increase in mortality from *DDT* could be explained by weight loss induced by the infection, and in fact comparable results have been produced by starvation alone (Dale *et al.* 1962). No interaction was observed when storage of *DDT* was relatively low, albeit higher than that observed in human populations.

Temperature and other environmental factors

The range of temperatures that people ordinarily encounter has little effect on oral toxicity, but increased temperature does cause increased dermal absorption of some compounds, for example, parathion (Funckes *et al.* 1963; Durham *et al.*

to be published). The maximal average increase is apparently in the order of 5 times. However, in man, the difference may be greater than 10 times for the first few hours of exposure and gradually decreases as exposure continues (Funckes *et al.* 1963).

Discussion

The fact that a number of factors influence dosage–response relationships should not obscure the fact that these relationships are real.

Table 10 summarizes part of the information in the foregoing paragraphs of this section. It is clear from this summary and from additional information in tables 8 and 9 that species differences may be more important than any factor except dosage in influencing toxicity. The largest ratio of difference found in connexion with species was over 230, while the largest ratio associated with any other factor was only 21. Furthermore, the numerical comparison ignores important phenomena that occur in man but are difficult or impossible to study in animals. One is forced to conclude that more emphasis should be placed on studies in man. This is particularly true when one considers that not only dosage and route but sometimes sex, age, temperature, duration, and other factors may be explored directly in volunteers or workers.

TABLE 10. SUMMARY OF INFORMATION ON THE IMPORTANCE OF DIFFERENT FACTORS INFLUENCING TOXICITY

factor	total no. of compounds	ratio of difference		increasing ratio indicates
		range	mean	
duration	23	0·5–20·0	—	2-year > *90-day
route	67	0·2–21	4·2	oral > dermal
species	20	0·2–11·8	1†	other sp. > rat
	1‡	> 230		other sp. > rat
individual§				
oral route	69	1·20–7·14	2·42	$LD50 > LD_1$
dermal route	42	1·37–14·93	3·00	$LD50 > LD_1$
sex				
oral route	65	0·21–4·62	0·94	male > female
dermal route	37	0·11–2·93	0·81	male > female
age	18	0·6–10·0	2·9	newborn > adult
	16	0·7–6·2	—	newborn > adult
	15	0·2–4·1	1·8	newborn > adult

* > indicates greater toxicity of chemical or greater susceptibility of animal.
† Approximate value.
‡ Norbormide.
§ Same sex.

OBSERVED INJURY FROM PESTICIDES

Let us turn now to a review of observed effects of pesticides in different human populations. In 1961, the mortality in England and Wales associated with acute poisoning by chemicals that can be used as pesticides was at the rate of 0·15 per million population (Anonymous 1963). All of the victims were adults. In five of the seven instances the compound involved could be used for a purpose other than pest control, and there was no way to determine with certainty whether a pesticide was involved or not.

The corresponding mortality rate for the United States of America was 0·65 per million based on separate investigation of each case, which was carried out through correspondence with the attending physician or medical examiner who had signed the death certificate (Hayes & Pirkle 1966). It was found that about 90 % of deaths attributed to pesticides were correctly diagnosed as indicated by evidence of adequate exposure, a consistent clinical course, and/or appropriate laboratory and autopsy findings. Of the reasonably definite cases, 51 % were in children under 10 years old; at least 58 % involved compounds older than *DDT*; not more than 15 % were occupational; and several cases were associated with alcoholic intoxication, mental deficiency, improper storage of poison, or some other special circumstance.

In the United States of America there has been no sustained trend toward increase or decrease in the mortality rate associated with pesticides. However, there has been an astonishing correspondence between the amount of pesticides used in different years and the number of deaths attributed in the same year to acute poisoning by pesticides (Hayes & Pirkle 1966). The limited use of pesticides in England and Wales may more than account for their relatively good safety record. In 1961, the number of deaths associated with all pesticides in England and Wales and in the United States, respectively, was 2·6 and 1·1 per thousand metric tons of selected pesticides. The compounds included the major organic phosphorus compounds, aldrin and related compounds, benzene hexachloride, *DDT*, nicotine, lead arsenate, mercurials, and the phenoxyacetic acid herbicides. These compounds were selected because they are important and because information was available on the weight of each used in the two countries (Farm Chemicals Handbook 1965; Strickland 1965; also in press). The weakness of the comparison is that of the seven deaths associated with pesticides in England and Wales in 1961 only two were caused by compounds (malathion and 2,4-D) which have no use except as a pesticide, and the other compounds (strychnine, trichloroethylene, arsenic, cyanide, and a mercurial) may have been intended for some other purpose. However, even on the basis of only two deaths, the mortality was 0·8 per thousand metric tons. Thus, the British safety record for pesticides is good in relation to population at risk and of questionable difference from that in the United States in relation to tonnage of pesticides used. The annual per capita use of the compounds mentioned above is 58 g in England and Wales, but 564 g in the United States.

However, other factors may be more important in determining safety than the amount of pesticide used. For example, I have pointed out elsewhere (Hayes 1964) that in 1961 a developing country had a mortality rate associated with poisoning by pesticides of greater than 2 per million population compared to 0·65 per million for the United States of America, whereas the annual per capita use of the organic insecticides selected for comparison in the two countries was 36 and 600 g, respectively. The mortality rate from pesticides is very high in some countries where the rate of use has not been reported but must certainly be no greater than that in the United States of America. For example, from the number of deaths reported we may calculate a rate of 4·1 per million for Israel (Wislicki 1961) and a rate of 5 for Japan (Namba 1961). The latter figure differs from all others mentioned above because it is restricted to parathion but involves an unknown proportion of suicides.

As we have seen, the relatively limited use of pesticides in England and Wales is an important factor in your good safety record, but this factor cannot account for the reported fact that children in England are almost entirely spared from severe injury by these compounds. Uniformity of language, education, and tradition must be contributing factors, but it is difficult to evaluate their importance or to determine whether they represent an adequate explanation. There may be other factors. Certainly, records of your poison control centre show that children are not completely spared from contacts with poisonous materials; in fact, children contribute 59% of the morbidity that is reported (Goulding & Watkin 1965). In the United States of America, children are involved in even a higher proportion (88%) of reports from poison control centres, and each year over half of those killed by pesticides are children under 10 years of age (Hayes & Pirkle 1966). The predominance of fatal poisoning in children has been noted in Israel (Arnan 1962), Australia (Clements *et al.* 1963; Isbister 1963; Phelan 1963) and Switzerland (Nouhen-Lang 1964), and it appears to be the usual pattern. I am sure you are interested in improving your record, but if you could also learn the secret of why your record is already so good—especially in relation to children—you might be able to give the people of our country and many other countries very welcome assistance.

In general, morbidity as well as mortality caused by pesticides is confined to people with occupational exposure or to others who have direct contact with formulations either through accident or suicidal intent. There have been isolated exceptions, and one of these was reported from Wales where about 150 people were made ill and approximately 30 had epileptic fits as a result of eating bread contaminated by endrin. The flour, and especially the bags that held it, had been contaminated with the insecticide by being shipped in a boxcar used earlier for shipping the poison (Davies & Lewis 1956). Somewhat similar episodes have happened in the United States and Canada where boys' pants were contaminated by mevinphos during shipment (Warren *et al.* 1963), and flannelette sheets were contaminated with parathion (Anderson *et al.* 1965). Children who used the clothing or who slept in contact with the sheets were poisoned. The contamination of food or clothing by pesticides during transport or shipping constitutes a real hazard. It has proved fatal in other countries (Karunakaran 1958; Kanagaratnam *et al.* 1960) and might easily do the same in our own. The hazard is one which is difficult to eliminate entirely unless it would be practical to reserve separate vehicles or separate sealed compartments in vehicles for the shipment of poisonous materials. You will recall that in the episode in Wales the flour was not shipped at the same time as the endrin; thus, prohibiting the shipment of poisons with food or clothing would not eliminate the danger completely.

On a worldwide basis, seed grain intentionally treated with organic mercury fungicides (Jalili & Abbasi 1961; Haq 1963) or with hexachlorobenzene, another fungicide (Schmid 1960; Cam & Nigogosyan 1963), probably has led to more human deaths than food contaminated by pesticides during shipment or storage. Apparently it is only through the vigilance of regulatory agents that injury from seed grain to man, and especially his domestic animals, has been avoided even in the developed countries.

EVALUATION OF HAZARDS

It is clear from the record that only people who absorb a relatively large dose of a pesticide are in danger of acute poisoning. Such people may include (*a*) those who are carelessly or accidentally exposed in the course of working with the compound, (*b*) children or other irresponsible people who ingest the material or have some other gross exposure to it, and (*c*) persons who are not irresponsible, but accidentally encounter a substantial amount of the compound in some completely unexpected way. I have just given some examples of the third type of accident. The problem in all three sorts of accidents is not so much an evaluation of the hazards, which are quite obvious after the fact, but in finding practical ways to eliminate them.

Most concern is expressed not about these obvious hazards, but about the possibility that repeated absorption of traces of a single pesticide or some combination of them will lead eventually to serious illness. It is sometimes implied that if such illness occurred, it would necessarily be chronic and would probably be obscure and difficult to diagnose. Actually, there is no necessary relationship between the number of doses and the chronicity of illness. Some materials, such as lead, triorthocresyl phosphate, or the carcinogens referred to in figure 3 can produce chronic illness from a single dose, whereas others, such as parathion, have so far produced only acute illness. In other words, with parathion the illness is similar whether it follows a single large dose or many somewhat smaller doses. If recovery occurs, it progresses at a rate determined by the severity of illness rather than by the number of doses which preceded it. Furthermore, as we have reviewed at some length, all of the various modalities of toxicity follow dosage–response relationships. Thus, if a certain daily dosage of a compound produces an injury, one can expect a larger dosage to produce the same injury in a shorter time. It is certainly true, as statisticians point out, that the number of observations necessary to detect a phenomenon dependably increases geometrically in inverse proportion to the frequency of the phenomenon. For phenomena that are rare, the necessary number of observations may exceed practicality. However, the difficulty can be overcome by observing the effects of high dosages. In experimental animals the dosage should be carried out to the point of causing death. Even in volunteers or workers, it is frequently possible to search for possible effects of dosages from 100 to 1000 times those encountered by the general population. If such dosages produce no detectable injury to the volunteers or workers, the chance that some injury to the general population will ultimately occur is extremely small, and, in fact, statistically negligible. Table 10 shows that in one instance injury was produced in rats fed for their lifetime at a dosage only 1/20th the dosage required to produce the same injury in other rats fed for only about 90 days, i.e. about one-eighth of their life span. This factor of 20 was the largest found for any set of conditions involving a particular species dosed by a particular route. I am not familiar with any drug or any other chemical which has produced illness in the course of a lifetime when given at a dosage one-twentieth of that which produced no effect when given for one-eighth of the life span of the species.

Time slips by so rapidly we tend to forget that *DDT* has now been commercially available for 20 years, and its military use is even longer. Twenty years is far more than one-eighth of the human life span. I venture to say that in some sectors of Naples where *DDT* was used so effectively to combat typhus in 1943 and 1944 (Simmons 1959), 20 years is a generation.

We have records of workers who have been employed in the formulation of *DDT* since 1945 or even earlier. These people remain well while absorbing dosages that for some are equivalent to 35 mg man^{-1}day^{-1} taken by mouth in solution (Ortelee 1958). Although one can find some reassurance in the fact that experimental animals tolerate even higher dosages without injury, the effect of such high dosages in man cannot be known with complete assurance until workers have spent their entire active lives in such employment. However, very substantial assurance about the prospects of the general population can be gained from consideration of these same workers. Exposure of the workers is approximately 200 times greater (Ortelee 1958) than the exposure of people who ate ordinary restaurant meals in the United States of America in 1953 and 1954 (Walker *et al.* 1954). Because of the decrease of *DDT* in our food (Durham *et al.* 1965), the factor is now only a little less than 1000. I have used figure 1 and the reported average storage of *DDT* in human fat samples collected in England and Wales (1·0 to 1·1 parts/10^6) (Egan *et al.* 1965; Robinson *et al.* 1965) to estimate that the average daily intake of *DDT* in England and Wales is slightly less than 0·02 mg man^{-1}day^{-1}. The absorption of *DDT* by many formulators is 1750 times this great. Even the absorption of *DDT* by the general population of one Indian city we have studied (Dale *et al.* 1965) is apparently 40 times greater than its absorption in England and Wales and 20 times greater than its absorption in the United States of America.

There is considerable advantage in approaching the question of safety through measurement of the storage or excretion of a compound or its metabolites. The reason is that either storage or excretion reflects total absorption. This approach avoids the error that might result from basing estimates of safety on measurement of exposure to materials which are actually never absorbed or a different error that might result from measuring exposure by a single route when one or more other routes were actually of importance. Measurement of storage or excretion also may have the advantage of being more sensitive than any other available measurements. Of course, the value of measurements of storage or excretion is increased when it is possible to learn the relationship between one of them and dosage in man as is done for *DDT* in figure 1.

Another approach to measuring the dosage of pesticides which people receive is to measure these compounds in food, particularly representative whole meals.

The use of meals rather than raw produce has the advantage that whatever cleaning, discarding of outer leaves, or cooking that might affect chemical residues will have been carried out. Therefore, measurements on prepared meals are more typical of true intake than are measurements carried out on food as sold in stores. Apparently the total diet in England and Wales has not been sampled for pesticide content. Measurements have been made of the pesticides in important foods (Report of the Government Chemist 1965). The concentrations found were higher for dieldrin and also in some instances for *DDT* than those reported for similar items in Canada (Swackhamer 1965) or the United States (Mills 1963; Williams 1964; Cummings 1965). However, the basis for selecting samples was so different that the comparison may not be valid. A comparison based on storage in human fat indicates about equal exposure to dieldrin but less exposure to *DDT* in England and Wales than in the other two countries.

Residues may be interpreted in terms of use levels (Miller 1965) or tolerances (National Agricultural Chemicals Association 1966) as in England and Wales, Canada, and the United States of America; or they may be interpreted in terms of permissible daily intake, as defined by joint committees of the World Health Organization and the Food and Agriculture Organization (FAO & WHO 1962; 1965*a*). In each instance, the level considered to be permissible has been based on a number of safety factors, some of which are used in calculation and some of which are not used in calculation but constitute a sort of bonus. The safety factor considered in every instance is some fraction (frequently 1/100) of a dosage that has produced no observed effect when fed to animals during their lifetime. Certain compounds have been studied in man, and this information may be taken into account. It may be astonishing to learn that at least 49 pesticides have been investigated in man. A table listing these studies has been given elsewhere (Hayes 1965). Some additional safety factors, which generally are not used in the calculation of safety limits, apply only to tolerances, for example, the fact that some residues may be reduced by cleaning, peeling, and cooking of food and the fact that not all food contains residues. Other additional safety factors apply equally to tolerances and permissible daily intakes, for example, the fact that a number of circumstances in agriculture tend to produce changes in the use of pesticides over a period of years. Exposure to a particular compound may last only one or a few seasons. Few pesticides will have such sustained value that there will be substantial exposure to them for even a single generation.

Documentation supporting both tolerances (Lehman 1965) and permissible daily intakes (FAO & WHO 1965*b*, *c*) have been published and should be consulted by anyone interested in the details. An important point which was brought out very clearly by the last joint meeting of the FAO Committee on Pesticides in Agriculture and the WHO Expert Committee on Pesticide Residues concerned with permissible daily intake was that our information on older pesticides, which we tend to take for granted, is vastly inferior to our information on the newer materials (FAO & WHO 1965*a*, *c*).

The discussion in this section has concentrated on *DDT* because it has had wider use in agriculture and public health and it has been studied more extensively in animals and man than the other chlorinated hydrocarbon insecticides, which now cause public concern. However, as shown by a recent review (Hayes 1966) all of these compounds have been monitored, and traces of many of them have been found in human food and in human tissues. Some of them, especially dieldrin (Hunter 1966) and methoxychlor (Stein *et al.* 1965), are being studied thoroughly in man. It is already clear that the same general conclusions will apply to them as apply to *DDT* although the exact factors of safety may be different. The safety of the chlorinated hydrocarbon insecticides seems especially great if it is compared to that of lead, which we seem to accept without concern. Kehoe (1961) showed that daily ingestion of 2 mg of lead per day (only about 6 times as much as occurs in the ordinary diet) produced a distinct continued increase in the concentration of lead in the blood and only 3 mg per day produced a more rapid rise in the blood lead concentration and the onset of mild symptoms of intoxication.

POSSIBILITIES FOR THE FUTURE

At least as early as 1954, Barnes & Denz (1954*a*) insisted on intensive study of a few animals of several species, an active and intelligent exploration of the pharmacological implication of the results, and relatively early extension of the investigation to man. I believe there is growing acceptance of this 'pharmacological' approach to toxicology, which stands in contrast to the routine 'life-time' study of large numbers of animals. There is also a growing awareness of the importance of tests in man (Medical Research Council 1964; Hayes 1965; National Academy of Sciences—National Research Council 1965).

There is no easy answer to the problem of safety. It has been suggested that new biological methods of pest control be developed not only to supplement but even to replace chemical methods of control. The suggestion is sometimes offered with an implication that use of biological methods would eliminate all difficulties. Experience shows that pests readily become resistant to biological controls; in fact, the existence of each species is evidence that through the course of evolution it has developed resistance against the various biological pressures brought against it. More important, determination of the safety of some kinds of biological control may be vastly more complicated than determination of the safety of chemical methods. For example, it has been proposed to use viruses for insect control. Until fundamental research on viral carcinogenicity is more advanced, I seriously doubt that anyone knows how to test a virus for safety. You will recall that a World Health Organization Group on Viruses and Cancer (WHO 1965) has stated: 'There seems to be reason to believe that future research will establish as a universal principle that [malignant] transformation is associated with the persistence in the cell of at least part of the virus genome and that transformation by virus is a direct result of the incorporation of virus nucleic acid in the cell.'

In spite of the difficulties, toxicologists whose main concern is for human health must turn more attention to naturally occurring toxins. The extreme carcinogenicity and hepatotoxicity of the aflatoxins has been demonstrated. Not enough work has been done to exclude the possibility that some other toxins of higher or lower plants have equally undesirable properties. It is only those chemicals man has encountered for generations that could explain the occurrence of diseases that some investigators suspect of being caused by chemicals even though their incidence has remained essentially stable.

The continuing need for pest control, the relative efficiency of chemical methods for achieving this control, and the degree of safety that has been maintained in the use of pesticides mean that use of many of these materials will continue for the predictable future. In the last few years, a tremendous amount of attention has been directed to certain real or potential side effects from the use of pesticides. In the United Kingdom, the use of aldrin, dieldrin, and heptachlor for seed dressing was discontinued because of evidence that seeds treated in this way killed birds (Great Britain, Ministry of Agriculture, Fisheries and Food 1964). Evidence was presented that seeds could be protected well enough in other ways. It was recognized that no danger to man was involved because of the vast difference in the circumstances of exposure and, consequently, in the dosage of aldrin, dieldrin, and heptachlor absorbed by man and by birds. At least for the time, uses of dieldrin were retained that produced results not readily achieved in other ways and yet involving small chance of side effects. This and other problems in the voluntary regulation of pesticides have been reviewed by Miller (1965). Adjustments are going to have to continue indefinitely no matter what kind of pest control is employed. At this time, there are certain aspects of residues and toxicity associated with the use of pesticides which we have not faced fairly although they are fundamental to present national and international regulatory systems. I refer to what have been euphemistically spoken of as 'zero tolerances', 'no residue' and 'no effect levels'.

The recent advances by which chemists routinely detect picogram quantities of a number of insecticides instead of milligram quantities have focused attention on the myth of 'no residue'. Although this phrase and the phrase 'zero tolerance' are generally associated with residues on food, I would like to illustrate my point with something a little closer to ourselves. It has recently been shown that people on both sides of the Atlantic store dieldrin in such a way that traces of it may be found in the blood of essentially everyone in the general population (Robinson 1963; Brown *et al.* 1964; Dale *et al.* 1966). The range found in all studies ($< 0{\cdot}0001$ to $0{\cdot}01$ parts/10^6) is similar to the range in each single study. The average values found for whole blood in different studies were 0·0012, 0·0025, and 0·0014 parts/10^6, respectively. The overall efficiency of the methods used is such that one can measure approximately 0·0001 parts/10^6. Any smaller concentration would be missed in analysis. Yet a fundamental principle of physical chemistry tells us that a single millilitre of blood from a person who happened to have 1/10 the smallest concentration of dieldrin that chemists can detect currently would contain (in view of the molecular weight) $1{\cdot}581 \times 10^{10}$ molecules of dieldrin. Certainly in spite of the miracles already achieved, analytical chemists have a long way to go. If one accepts Avogadro's rule, one must abandon the concept of no residue or zero tolerance. Instead, the concept of negligible residue must be adopted. There must be some level at which biological significance is lost.

When we consider biological effects we lack the corresponding facts and the neat formulation of them implied in Avogadro's law. However, logic still has a place in science. Is it conceivable that a single molecule in the body is completely without effect? Pharmacologists, like chemists, have made increasingly refined measurements of the effect of drugs. No doubt the trend will continue. As more and more refined observations are made, we will gradually acquire more biochemical details on how chemicals work, but an increasing proportion of the observations will not involve injury.

To an ever growing extent, toxicologists must assume responsibility for deciding whether observed changes involve injury or signify adaptation. Toxicologists must also assume more responsibility for declaring certain things to be safe. It is no longer enough to use the best available methods to identify and to avoid danger. Toxicologists must also assume responsibility for identifying safe uses of chemicals and making the information freely available to those who need it. It was once supposed that solvents were rapidly lost from the body following ordinary occupational exposure to them. However, the use of new analytical methods has shown that some of them such as tetrachloroethylene and carbon tetrachloride may persist for periods of days to weeks depending on the dosage (Stewart *et al.* 1961, 1965). Must we, as a result of this new information on persistence, suppose that workers exposed to industrial solvents are now in more danger from this source than were their fathers who worked in the same plants? Obviously this would be absurd, and yet the only possible way to avoid having at least some people absorb at least a trace of any given chemical is to abandon its manufacture. In connexion with many pesticides, the people involved are the whole population of the world. No one would seriously consider the idea of abandoning the older chemicals, which have a firm place not only in industry but on our farms and in our homes. What is needed is a more rational way of introducing new chemicals regardless of their use. Toxicologists have just as much responsibility to advise of safety as they have to warn of danger.

SUMMARY AND CONCLUSIONS

All modalities of toxicity exhibit a dosage–response relationship. Since the quantitative importance of various factors—other than dosage and occasionally species and sensitivity—that modify the toxicity of a given compound is limited, it follows that real assurance about the possible long-term effect of small repeated doses may be gained by studying the effects of larger doses given over a briefer period. This is particularly true if the potential problem of important species difference can be overcome by studies in volunteers and in workers whose exposure is more intensive and often more continuous than that of the general population. It is especially among such workers that a meaningful search may be made not only for the recognized effects of a particular compound but also for effects that are suspected but undemonstrated.

Any compound may present a hazard if it is misused, and the modern pesticides are no exception. However, there is strong and growing evidence that long-term

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absorption of traces of one or more of the synthetic nonmetalic pesticides will not lead to illness.

More emphasis should be placed on what may be called the pharmacological approach to toxicology, that is: intensive study of a few animals of several species, active and intelligent exploration of the pharmacological implications of the results, and relatively early extension of the investigation to man.

Toxicologists must keep alert as the introduction of new and perhaps nonchemical methods of pest control introduce new and perhaps more difficult problems of determining safety. At the same time and in view of the ever increasing refinement of chemical and pharmacological measurements, toxicologists must recall that their final responsibility is just as much to advise of safety as to warn of danger.

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TOXIC HAZARDS OF SOME POISONS

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Savannah, Georgia

The article presents statistics

It is of great importance that we achieve a balanced view of the problem before us. This may be done in part by reviewing certain statistics on fatal chemical poisoning. Table I reveals that chemical poisoning occupies a minor place as a cause of death. Accidents of a mechanical nature are more common. Table II shows that there has been no significant change in the rate of fatal chemical accidents during the last decade. This may be somewhat surprising when one considers the rapid increase in the number and tonnage of toxic compounds which has taken place during this same period. The quantity of a few pesticides produced in the United States is shown in Table III. Table IV shows that deaths caused by arsenicals and a few of the other older pesticides, chosen for purposes of illustration, continue to occur at about the same rate as they did in 1940 before the introduction of the newer pesticides. This is explained by the fact that the newer toxicants have not so much supplanted as supplemented the older ones.

The tables include:

TABLE I.

Deaths, per 100,000 population, from selected groups of causes and crude death rates. United States, 1948.[2]

Cause	Number	Rate
All causes	1,444,337	988.5
Infectious and parasitic disease	73,016	50.0
Cancer and other tumors	202,186	138.5
Disease of the circulatory system	505,716	346.1
Chronic poisoning and intoxication	2,550	1.7
Alcoholism	2,433	1.7
Lead poisoning	46	0.0
All other chronic poisoning	71	0.0
Violent or accidental deaths	123,009	84.2
Accidental deaths	98,001	67.1
Automobile accidents (except collisions with trains or street cars)	29,915	20.5
Accidental absorption of poisonous gas	2,002	1.4
Acute accidental poisoning by solids and liquids	1,436	1.0

TABLE II.

Total accidental deaths in the United States caused by chemicals and rate per 100,000 population.[2]

	Gases		Solids and Liquids	
Year	Number	Rate	Number	Rate
1939	1,396	1.1	1,371	1.0
1940	1,502	1.2	1,324	1.0
1941	1,464	1.1	1,191	0.9
1942	1,694	1.3	1,193	0.9
1943	2,038	1.5	1,254	0.9
1944	1,907	1.4	1,381	1.0
1945	2,131	1.6	1,532	1.2
1946	1,874	1.3	1,536	1.1
1947	1,938	1.4	1,504	1.0
1948	2,002	1.4	1,436	1.0

The relatively good safety record of the older economic poisons is due in part to the fact that workers are aware of their hazard and have learned to treat them with respect. Unfortunately, a part of their reputation for safety depends on a passive acceptance of a small but continuing series of accidents. This same kind of careless thinking may lead one to ignore the importance of common household chemicals and non-prescription drugs as a source of danger. Accidents associated with these materials are much more frequent than those caused by economic poisons. A home accident survey conducted by the Accident Prevention Service, District of Columbia Chapter, American Red Cross, has illustrated this over a period of years. Of 287 cases of fatal and nonfatal chemical poisoning in which a definite cause was listed in 144 instances, six were caused by insecticides and eleven by rodenticides, while at least 22 were ascribed directly to kerosene and 31 to aspirin. Another good illustration of the danger of household chemicals is the study by Rubin and his colleagues,[4] who showed that among 250 consecutive admissions for the ingestion of poisons, 21 percent of the children studied had taken kerosene, while only 9 percent had taken one of a variety of pesticides.

TABLE III.

Estimated production of selected pesticides in the United States, expressed in thousands of pounds.[3] and

Year	Calcium Arsenate	Lead Arsenate	DDT	BHC
1939	41,349	59,569		
1940				
1941	56,136	75,912		
1942	84,136	63,751		
7943	74,854	73,955		
1944	44,350	90,705	9,626	
1945	25,644	79,522	33,243	
1946	35,392	56,667	45,651	
1947	46,904	31,006	49,600	8,197
1948	27,234	24,630	20,240	18,382
1949	16,006	16,866	37,904	27,937
1950	49,474		77,973	57,509

TABLE IV.

Number of accidental deaths in the United States from selected causes.[2]

Year	Arsenic and its Compounds	Nux Vomica and Strychnine	Tobacco and Derivatives	Cresol Compounds	Lye and Potash	Barbituric Acid and Derivatives
1939	109	82	7	31	120	201
1940	73	67	7	32	111	246
1941	70	40	7	29	94	232
1942	71	50	12	25	87	197
1943	85	54	6	20	88	226
1944	77	54	12	17	74	270
1945	58	52	9	13	79	392
1946	63	45	11	19	90	436
1947	48	37	10	7	72	418
1948	63	30	8	8	74	419

The facts which have just been mentioned we believe, should be viewed as a challenge to the medical profession and the pesticide industry to reduce these preventable, and therefore unnecessary, accidents. The solution of the problem will, in the long run, be benefited by a balanced perspective. Scare tactics may create a flurry of intense activity and may result in the correction of real abuses. However, there is such a thing as crying "wolf" too often, and those who resort to scare tactics frequently may end by losing the sheep and the shepherd boy as well.

New Pesticide Accidents

A brief review of the accidents associated with the newer pesticides may now be made. Thirteen fatal accidents have been clearly associated with sodium monofluoroacetate (1080) and five other deaths may have been caused by it. There

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[1] Communicable Disease Center, Public Health Service, Federal Security Agency.

[2] U. S. Public Health Service. Vital Statistics of the United States Pt. I 1939-48.

[3] Production statistics based on U. S. Tariff Commission and Bureau of Census figures.

[4] *Clin. Proc. Children's Hosp.* Washington, 5(3): 57-73, February, 1949.

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have been at least six nonfatal cases. The established fatalities have varied from one to five per year since 1946. Although figures are not available regarding production or sale of the compound, it is clear that the amount is small as compared with some of the other pesticides, including parathion. Furthermore, it is only because of the unusually strict controls voluntarily imposed by the manufacturer that the record has been kept as good as it is. It is possible that the introduction of warfarin, a relatively safe rodenticide, may lead to a more restricted use of 1080 and a continuation of the decrease in accidents caused by it—a decrease which seems to have been present for at least a year.

ANTU has a good safety record for man in spite of the fact that at least ten children are said to have eaten baits containing the rodenticide.[5] There remains a need for a fast-acting rodenticide that is safe for man and effective against all species of commensal rodents. Although the new slow-acting rodenticides have a good safety record, there remain rodent control problems which are more conveniently solved by a fast-acting rodenticide.

Among the newer insecticides, the organic phosphorus compounds have produced by far the most fatal accidents. Parathion has accounted for eight deaths in the United States, and there was one other in which the part played by parathion could not be evaluated clearly. A large number of nonfatal cases is known, and the majority is probably never reported. However, the very active educational campaign carried out by the manufacturers has done a great deal to bring information to the farmers and orchardists who buy and use the product. This campaign, along with the efforts of private, state, and federal agencies, has apparently reduced the accident rate, although use of parathion has increased. Industry is to be congratulated for its part in promoting safer practices in the use of economic poisons.

Tetraethylpyrophosphate (TEPP) has caused at least nine fatal accidents, and there are two other cases in which the diagnosis is not established.

Among the chlorinated hydrocarbon insecticides, chlordane is known to have killed one factory worker. In a second case, the diagnosis is in doubt.

Some fourteen deaths have been listed as resulting from DDT intoxication. All but two of them involved ingestion and, in most, the action of the solvent in the formulation undoubtedly played an important if not deciding role. Two fatalities ascribed to DDT involved *agranulocytosis* and *periarteritis nodosa*. The diagnosis of DDT poisoning in these instances stands on the weakest of foundations.

It is, of course, recognized that there are usually several nonfatal accidents for every fatal one. The reporting of the fatal ones is much better, however, and they serve as a very good indicator of the seriousness of acute poisoning in general.

Chronic poisoning may, in specific instances, be much more significant than acute poisoning. Chronic intoxication is recognized in our vital statistics. As shown in Table 1, approximately two thousand five hundred cases are reported annually. Alcoholism accounts for the greater proportion, while the remainder—about five percent—includes poisoning by lead and a wide variety of other materials. At the present time, cases of chronic poisoning in man by the newer pesticides have not been confirmed unless one lists in this category certain cases involving occupational exposure to organic phosphorus insecticides. If one may judge from animal experiments, intoxication following repeated exposure to small doses of the newer pesticides would be similar to illness caused by a single large dose. Such a relationship does not hold for all chemicals; for example, chronic and acute lead poisoning may be quite dissimilar. We must, therefore, be on the alert for syndromes characteristic of chronic illness. Furthermore, we must remember that man, in addition to being able to report his symptoms, may show different signs of illness than are shown by laboratory animals.

Advice on Poison Cases

In this connection, a word of caution is needed. While remaining on the alert for exceptions, we cannot afford to forget the general rules. Any case of human illness ascribed to poisoning should be accompanied by convincing evidence of excessive exposure and by evidence that the signs and symptoms resemble those known to be characteristic. If this cannot be done, a diagnosis of specific intoxication should not be made unless every effort has been made by competent specialists to rule out other causes. Careful attention should be given to the principles of epidemiology. In general, those persons with the greatest exposure to a compound will show the greatest effects—if effects are to be shown. This is true even of those materials, such as beryllium, in which there is good evidence that a sensitization reaction constitutes a significant part of the mode of action.

A word of caution is also in order regarding the expression "chronic toxicity." In simple terms, the expression means prolonged poisoning. It is generally caused by repeated small exposures to a toxicant but, in certain instances, it may be caused by one or a few large doses, as with arsenic. Conversely, small repeated doses may result in acute poisoning. An example is poisoning by small, repeated doses of parathion, in which the cholinesterase level is successively lowered until it reaches a point at which symptoms of acute poisoning occur.

In conclusion, the newer pesticides have, in comparison with the older ones, a good safety record. This is in large part due to intelligent enforcement of the Federal Insecticide, Rodenticide, and Fungicide Act, and of similar legislation in the various states. Educational campaigns carried on by a great variety of groups, including industry, have helped tremendously to familiarize users with the facts and to establish the custom of reading the labels on economic poisons. The American Medical Association, The U. S. Public Health Service, and others have called the attention of physicians to the symptomatology and treatment of intoxication caused by various pesticides.

Since a great many new pesticidal compounds have been introduced in the last decade, it is obvious that many problems require further scientific study. There is nothing to be gained in the long run, however, by irresponsible statements that nothing is now known of the toxicology of the newer pesticides, or that no legal control of their use exists, or—in the absence of epidemiological proof—that a wide variety of illness from which mankind has suffered for generations is now caused by intoxication by the newer economic poisons.

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[5] Emlen, J. J.: *Amer. J. Pub. Health*, *37*(6): 721-727, June, 1947.

AIMS AND PURPOSES OF THE ASSOCIATION OF ECONOMIC POISONS CONTROL OFFICIALS

By ALBERT B. HEAGY, Secretary
College Park, Md.

I wish to express my appreciation to your Chairman for this opportunity to appear on your program on behalf of the Association of Economic Poisons Control Officials. It was your Chairman's wish that I tell you something of our Association for the benefit of those of you who have not previously had the opportunity of meeting with us.

Historically, this group was organized during a meeting of the Western Cooperative Spray Conference in Portland,

ATTENTION

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The following abstract is one from the symposium entitled: "Metabolic Reactions to Toxic Substances" co-sponsored by the Society of Toxicology and the American Society for Pharmacology and Experimental Therapeutics, Inc. held Tuesday, August 28, 1962 at Nashville, Tennessee.

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THE TOXICITY OF CHEMOSTERILANTS

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It is possible to show by mathematical models that sterilization of males is ultimately more efficient than killing as a method of controlling insects and perhaps other pests. The reason is that although both dead and sterile males fail to reproduce, some portion of the sterile ones will effectively prevent the reproduction of females that were not directly affected by the sterilizing agent. The practicality of the technique was demonstrated in a series of brilliant experiments with the screw-worm fly (*Callitroga hominivorax*) sterilized by radiation. The species was first eradicated from the Island of Curacao and later from Florida and the Southeastern States. An effort is being made now to broaden the method so that sterilization can be produced by a chemical rather than by radiation. A chemical used in this way is called a chemosterilant. The chemicals that have already shown significant value in laboratory tests fall into three classes: alkylating agents, antimetabolites, and miscellaneous compounds. So far, the most promising compounds are alkylating agents. For this reason, this abstract is confined to the toxicology of these compounds.

Many alkylating agents, including most of those considered as chemosterilants, have little immediate pharmacologic action. The entire class is notable for its selective action against some proliferating tissues and certain other cells. In general, the blood is the most susceptible tissue, but there can be no assurance that more sensitive tissues or functions will not be found. Different compounds show some specificity against different cell types. A number of alkylating agents have been shown to be mutagens in insects, bacteria, fungi, and higher plants; carcinogens in mammals; and teratogens in insects, birds, and mammals. Some, but not all, produce sexual sterility in insects and mammals. Some have an established reputation as drugs for treating leukemia and other neoplasms. All of these actions resemble the effects of radiation, but the more careful the study, the more differences in detail have been found. Evidence has been presented that alkylating agents and ionizing radiation both attack DNA but by different chemical reactions. Even if this is true, the differences may be more important than the similarities. In comparison with common insecticides, the candidate chemosterilants are of high to moderate toxicity. Their metabolism is rapid and extensive so that the compounds show minimal storage. Whether they will have cumulative effects on mammals when absorbed at dosages likely to be encountered remains to be learned.

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Organic Phosphorus Poisoning and Its Therapy

PUBLICATION

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With Special Reference to Modes of Action and Compounds That Reactivate Inhibited Cholinesterase

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Introduction

Organic phosphorus compounds are of considerable interest and importance by virtue of their widespread use as insecticides; their effectiveness in the treatment of myasthenia gravis, glaucoma, and abdominal distention; and their potential application as war gases. These compounds owe their pharmacologic effect primarily, if not entirely, to their ability to inhibit the enzyme cholinesterase with a resultant overstimulation of the parasympathetic nervous system by the acetylcholine which accumulates. Atropine has been the drug of choice for the treatment of organophosphorus poisoning. This therapy produces relief of symptoms based on blocking the action of excess acetylcholine.

More recently, a number of specific antidotes have been developed which act to repair the basic biochemical lesion in organic phosphorus poisoning by freeing the cholinesterase from its combination with the inhibitor. In the present paper, some of the pertinent background material relative to organic phosphorus poisoning will be reviewed, and the properties and usage of these specific antidotes will be discussed in some detail. Emphasis will be given to those aspects of symptomatology and treatment which might be of most value to the physician faced with a presumptive case of poisoning.

Nature and Physiologic Function of Cholinesterase

There are 2 general types of cholinesterase present in the animal organism.[9] These are: (1) the true, specific enzyme, acetylcholinesterase, which has an almost specific affinity for the naturally occurring substrate acetylcholine, although it will hydrolyze several synthetic esters; and (2) the nonspecific enzyme, pseudocholinesterase, which has the ability to hydrolyze quite a wide range of naturally occurring and synthetic esters in addition to acetylcholine. Acetylcholinesterase occurs in the nervous system, where it is of great functional importance; in muscles; and in glands; and it also occurs incidentally in erythrocytes. The pseudoenzyme is found in the blood plasma and in various tissues, including the central nervous system.

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From the Toxicology Section, Technology Branch, Communicable Disease Center, Public Health Service, U.S. Department of Health, Education, and Welfare, Wenatchee, Wash. (Dr. Durham) and Atlanta (Dr. Hayes).

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In addition to location and substrate specificity, there are other differences between true and pseudocholinesterases. The latter functions well in the presence of an excess of substrate, while the former is inhibited under this condition. A number of inhibitors show considerable specificity for one type of enzyme. For example, diisopropyl fluorophosphate (DFP) in low concentrations is almost completely specific for pseudocholinesterase.[73]

It has been suggested that certain direct effects of the anticholinesterase organic phosphorus compounds do not depend on inhibition of cholinesterase.[105] Von Kaulla and Holmes [142] have reported that patients poisoned by organic phosphorus compounds show abnormalities in the blood-clotting mechanism, but the changes were in both directions from normal and must be considered unconfirmed. Some of the compounds do produce a delayed reaction similar to "Jake-leg paralysis," [11,41,94] but the clinical picture is quite distinct from that of ordinary poisoning. Although any statement about the relationship between cholinesterase inhibition and the classical picture of poisoning must be qualified in terms of the rate of depression, the degree of depression, and perhaps other factors, the evidence for a *lack* of relationship is indefinite. On the contrary, the evidence for a *lack* of relationship between cholinesterase inhibition and paralytic effect is much more clear. In spite of these exceptions, it is true that the predominant pharmacologic effect of the organic phosphorus compounds is inhibition of the enzyme acetylcholinesterase.

In order to have an understanding of the action of acetylcholinesterase and of the effect of its inhibition on the animal organism, it is first necessary to look at the physiologic action of acetylcholine. Acetylcholine is the chemical mediator of the parasympathetic nervous system and is necessary for transmission of the nervous impulse (1) from preganglionic fibers to autonomic ganglia; (2) from postganglionic, cholinergic nerves to smooth and cardiac muscle and to secretory cells: (3) from motor nerves to striated muscle; and probably (4) from certain structures within the central nervous system. Normally, in the presence of the usual concentration of cholinesterase, the acetylcholine formed during the process of transmission is hydrolyzed almost instantly so that the synapse is again ready to transmit a physiologic impulse. A small abnormal accumulation of acetylcholine at the synapse or myoneural junction produces an abnormal increase in function (e.g., fasciculation of muscle), while greater accumulation rapidly produces a decrease in function (e.g., paralysis). A decrease in cholinesterase activity is accompanied by an accumulation of acetylcholine. The symptoms caused by organic phosphorus poisoning are, therefore, very similar to those resulting from overstimulation of the parasympathetic nervous system.

Symptoms of Organic Phosphorus Poisoning

Absorption of the organic phosphorus anticholinesterases may occur through the lungs, gastrointestinal tract, or skin. Absorption of these materials is more rapid and more complete through the former 2 routes than through the latter. Respiratory exposure may be of predominant importance wherever there is a sufficient concentration of vapor or of aerosol fine enough to inhale. However, in many agricultural and public health usages, workers get contamination predominantly on their skins.[13,30,42] The oral route of exposure occurs in accidental poisoning (particularly of children), in murder, and in suicide. Ingestion has not been considered of importance in occupational exposure; but a case has been reported in which a parathion sprayman became severely poisoned, presumably from eating a candy bar which he had been carrying for 6 hours in an open outside pocket of his work clothes.[120]

Although many of the organic phosphorus compounds have a high acute toxicity, agricultural residues of these materials on food have not been a problem, due to their rapid breakdown and the fact that they are not stored in the animal body.[40] Direct contamination of food by concentrated formulations of these insecticides during shipment has been the cause of several outbreaks of poisoning in other countries.

The symptom picture in organic phosphorus poisoning may vary in severity, in rapidity of onset, in duration, and in range, depending upon the route and the magnitude of exposure. Minor exposure to a vapor or aerosol of a direct inhibitor of cholinesterase may produce local effects on the eye or respiratory system through local absorption and without systemic effect. The optic effects consist of miosis, a sensation of pressure in or behind the eye, headache, and conjunctival hyperemia. Unilateral manifestation of these optic effects has been implicated in visual difficulties experienced by pilots applying these materials.[140] The local effects on the respiratory tract involve increased secretion, a feeling of tightness in the chest, and occasionally wheezing. Localized massive dermal exposure can lead to muscular fasciculation and sweating confined to the area of absorption.

Systemic effects may follow absorption by any route. If there has been adequate exposure to a vapor or aerosol, the local respiratory effects already described will appear, but they will be rapidly followed by more severe optic and respiratory distress and by systemic manifestations. Dixon[88] has pointed out that too much reliance should not be placed on miosis as a diagnostic sign, since some poisoning cases, at least early in their course, do not exhibit miosis, and a few even have mydriasis.

In systemic poisoning, the muscarine-like effects are usually first to appear. They include anorexia, nausea, sweating, epigastric and substernal tightness (probably due to cardiospasm), heartburn, belching, and tightness in the chest. The sequence of symptoms varies somewhat with the route of exposure—gastrointestinal effects usually being earliest after ingestion; sweating, and at times muscular fasciculations, after dermal exposure; and respiratory effects after inhalation. More severe exposure by whatever route produces abdominal cramps, increased peristalsis, vomiting, diarrhea, salivation, lacrimation, profuse sweating, pallor, and dyspnea. In some subjects there is audible wheezing. More severe signs and symptoms include involuntary defecation and urination, excessive bronchial secretions, and (according to some authors) pulmonary edema.

Nicotine-like effects appear usually after the muscarine-like effects have reached moderate severity. These include muscle twitching, fasciculations, and cramps. At about the same time, there appears increased fatigability and mild, generalized weakness which is increased by exertion. Extensive exposure produces severe weakness, including weakness of the muscles of respiration. There may be a mild or moderate elevation of blood pressure.

The central-nervous-system effects include tension, anxiety, restlessness, giddiness, and emotional lability. Late effects include insomnia, excessive dreaming, and occasionally nightmares. Greater exposure produces headache, tremor, drowsiness, difficulty in concentration, slowness of recall, and confusion. There may be withdrawal and depression. In the absence of symptoms, there is no change in the electroencephalogram (EEG).[66] Mild symptoms are accompanied by a slight diminution in potential, and moderate symptoms are accompanied by irregularity of rhythm, variation and increase of potential, and bursts of abnormal waves more or less reminiscent of waves seen in epileptics.[157] Lethal or near-lethal doses produce ataxia, slurring of words, multiple repetition of the last syllable of words, coma, areflexia, Cheyne-Stokes breathing, and finally, respiratory arrest.

The cause of death may usually be attributed to interference with respiration.[66] Animal experiments have proved that the anticholinesterase organic phosphorus compounds interfere with respiration in at least 4 ways, including bronchoconstriction, excessive respiratory secretion, failure of the muscles of respiration, and depression of the respiratory center. Although the respiratory center is at first stimulated by anoxia of whatever cause, it is rapidly depressed by continued anoxia. The severe bronchial constriction seen in animals following exposure to parathion or nerve gas has not been seen in man.[60] The difference may depend on the different amount of bronchial musculature in different species.

The compounds also produce bradycardia and various degrees of A-V block. If these effects are prevented by atropine, and if the animal is prevented from a respiratory death by artificial respiration, it will eventually succumb to a sufficient dose through dysfunction of the heart. This dysfunction will take the form of ventricular fibrillation if atropine should be administered in the presence of severe anoxia.[137] Even if atropine is given correctly, bradycardia and perhaps impaired contractility appear shortly before asystole in the animal maintained by artificial respiration.[83] Unanesthetized dogs, unlike anesthetized ones, exposed to sarin by various routes generally show bradycardia, increase in pulse amplitude, decrease in oxygen tension, increase in carbon dioxide tension, and fall in arterial blood pH before changes in respiratory rate and volume are evident.[58,59]

The local effects of organic phosphorus exposure begin within a few minutes after exposure and last for periods ranging from several hours to a day, except that miosis may last 2 to 5 days or occasionally longer. Moderate systemic effects begin within about half an hour after respiratory exposure, three-quarters of an hour after oral exposure, and 2 to 3 hours after dermal exposure. With sufficient exposure, the onset of symptoms is essentially instantaneous, with death in a few minutes. Moderately severe symptoms may not reach their maximum until 4 to 8 hours after onset; they diminish over a period of 1 to 6 days. However, EEG changes may persist for 11 to 18 days.[66]

Mechanism of Inhibition of Cholinesterase

In recent years, much has been learned about the molecular forces involved in the interaction of acetylcholinesterase both with its natural substrate and with organic phosphorus inhibitors.[111] Wilson and Bergmann [133] have postulated that the surface of the enzyme protein contains 2 active centers, an "anionic site" and a "cationic or esteratic site." The former, by means of coulombic forces, attracts the positively charged quaternary nitrogen atom of acetylcholine and thereby fixes and orients the substrate in a proper position so that the esteratic site can exert its hydrolytic effect. The esteratic site is thought to consist of a hydrogen-bonding group which anchors the ester end of the acetylcholine molecule. It has been suggested that the ester-anchoring group may be the iminazole ring of histidine and that any phosphoryl group which becomes attached here is subsequently transferred to an adjacent serine moiety of the enzyme molecule by a $N \rightarrow 0$ shift. It is thought that the effect of change in pH on the hydrolytic activity of this enzyme is mediated through changes in ionization at these 2 sites.

In the following stage of the physiologic reaction, the acetylcholine is split into choline which is set free and the acetyl moiety which remains affixed to the enzyme. However, the acetylated enzyme is unstable and easily breaks down to form active enzyme and acetic acid.

The reaction between enzyme and organic phosphorus inhibitor is thought to involve only the esteratic site, except with the phosphorylcholines [57] that are used as laboratory tools only and need not be discussed further. This center is phosphorylated to form a complex which is, at least by contrast with the acetylated enzyme, quite stable. The phosphorylation is thought to proceed in 2 stages, as discussed in detail below under "Chemical and Physiologic Properties of Oximes." The stability of this phosphorylated enzyme accounts for the fact that organic phosphorus compounds are inhibitors rather than substrates for cholinesterase. During this inhibition reaction, the anionic site remains uninvolved.

Measurement of Cholinesterase Activity and Its Relationship to Symptomatology

Cholinesterase activity can be determined by any of several methods based on: (1) direct or indirect measurement of the acetic acid or other moiety released by hydrolysis of acetylcholine or other esters, or (2) direct measurement of acetylcholine remaining after partial hydrolysis.

The electrometric method of Michel[107] together with a micromodification of this technique for use with capillary blood samples[104] is probably the most widely used method. There are also available colorimetric,[108] titrimetric,[10] and manometric[1] procedures. The colorimetric screening method of Limperos and Ranta[100] is adaptable to the approximate determination of large numbers of samples under field conditions. Other procedures involve use of the Hestrin method[75] for measurement of acetylcholine.

The restoration of plasma or of plasma and red blood cell cholinesterase activity may be increased by plasma or whole blood transfusions in animals without any effect on the symptoms of poisoning or appreciable reduction in the susceptibility to further exposure.[14]

Although the acetylcholinesterase of red blood cells and the more general esterase of the plasma are not directly related to the signs and symptoms of organic phosphorus poisoning, these enzymes are inhibited in a manner essentially parallel to the inhibition of acetylcholinesterase of nerve, muscle, and gland. The blood enzyme levels, and particularly that of the erythrocytes, may be used as an index of tissue enzyme levels. The erythrocyte cholinesterase level has been shown to correlate rather closely with the activity of this enzyme in the brain during both poisoning and recovery in the rat.[60] Following a single exposure, maximum depression of blood cholinesterase occurs within a few hours. The degree of depression varies with the amount of absorption, the logarithm of the fraction of enzyme inhibited being proportional to the amount of toxicant absorbed in connection with essentially instantaneous dosage. Of course, absorption is influenced by route, often being prolonged for hours or even days after skin exposure.

Multiple exposures over a brief period are partially cumulative in their effect. Experience suggests that small multiple exposures over an extended period are not indefinitely cumulative in their effects but that the cholinesterase level reaches a plateau.[74,135] There is some evidence that the rate of inactivation of tissue cholinesterase, as well as the degree of inhibition, may influence the level of tissue enzyme activity at which symptoms begin. The cholinesterase activity of the red blood cells may be gradually depressed to near zero by repeated exposure over a period of weeks without systemic symptoms necessarily ensuing, or without any relation to the severity of symptoms that occur.[127,135]

Following exposure, the blood enzymes, especially the erythrocyte cholinesterase, remain at a low level of activity for some time after the disappearance of symptoms. This lag is thought by some to indicate that cholinesterase activity is restored more rapidly in the tissues than in the blood, although the tissue regeneration rate is not fully known. Chronic-feeding and certain other studies indicate that the phenomenon may be accounted for by tolerance rather than by a difference of recovery rate for different enzymes.[127] The adaptation to constant concentrations of acetylcholine that has been demonstrated to occur in ganglia[98] may well occur in other parts of the nervous system. This adaptation almost certainly contributes to the lack of parallelism between symptomatology and cholinesterase level.

It is true that effects of doses of anticholinesterase compounds near the fatal level are partially cumulative if the doses are repeated at relatively brief intervals. Thus, Callaway and Davies[21] have found in rabbits and guinea pigs that lowering the blood cholinesterase level to 50% of normal by either a single dose or repeated doses of tetraethyl pyrophosphate (TEPP) or sarin results in an increased susceptibility of the animal to these agents.

Plasma cholinesterase is formed by the liver. Following cessation of exposure, plasma enzyme activity is increased by about 13% of original activity during the first day, and more slowly thereafter, so that 30 to 40 days are required to reach the normal pre-exposure level.

Once fully inhibited, the enzyme content of a particular erythrocyte is not regenerated. Rather, in the absence of treatment with oximes, the rate of regeneration of red-blood-cell cholinesterase reflects the replacement of red corpuscles in the circulation and thus requires 90 to 100 days to return to original activity after nearly complete depression. Regeneration occurs at a regular rate of about 1% of normal per day.

Barstad [18] concluded that respiratory function should be only slightly impaired by a peripheral cholinesterase inhibition approaching 90% and that consequently a rather slight degree of cholinesterase reactivation at the critical site should be sufficient to relieve the peripheral failure of respiratory movements during poisoning with anticholinesterase agents.

Measurement of Metabolites and Their Relationship to Symptomatology

Exposure to those organic phosphorus compounds which, on hydrolysis, form *p*-nitrophenol or one of its congeners can be estimated by determination of these phenolic compounds in urine.[45] Although applicable to a restricted group of compounds, this test has proved to be a more sensitive measure of absorption than is cholinesterase inhibition.[4] A urine test based on excretion of organically-bound phosphorus would be desirable, since metabolites of this type are, of course, common to all the compounds in this group.

Use and Action of Atropine and Other Nonspecific Antidotes

Until recently, the most important factors influencing survival after otherwise lethal cholinesterase inhibition appear to be prompt administration of atropine in sufficient dosage and artificial respiration, if required.[61]

The suggested dose of atropine is 2 mg. initially, and as much as 6 mg. may safely be given within 10 minutes or more without medical supervision. Individuals poisoned by anticholinesterase organic phosphorus compounds have an increased tolerance for atropine. Furthermore, a single dose of as much as 10 mg. of atropine has been inadvertently administered intravenously to normal adults without endangering life, although it has, of course, produced very marked signs of overdose. In the presence of severe anticholinesterase poisoning, 40 mg. of atropine may be given in a day without producing symptoms of overatropinization. The low toxicity of atropine and its effectiveness in the treatment of anticholinesterase intoxication have been discussed by Gordon and Frye.[65]

Severe symptoms of organic phosphorus poisoning should be treated by the physician with the intravenous injection of 2 to 4 mg. of atropine. The effects of intravenous atropine begin in 1 to 4 minutes and are maximal within 8 minutes. If muscarine-like symptoms are not relieved, and if signs of atropinization (dry, flushed skin and tachycardia) do not appear, the intravenous injection of atropine in doses of 2 mg. should be repeated at 5- to 10-minute intervals until symptoms are relieved or signs of atropine overdosage appear. A mild degree of atropinization should be maintained in all cases for 24 hours and in severe cases for at least 48 hours.

Atropine should not be given to an anoxic patient because of the danger of producing ventricular fibrillation. In the cyanotic patient, artificial respiration, oxygen, or other indicated measures should be carried out first to correct the anoxia, and then atropine should be given.

Atropine should not be administered for preventive purposes in persons who anticipate exposure to anticholinesterase agents. Its use in this manner may mask the early occurrence of signs and symptoms of intoxication and allow the patient to expose himself to dangerous levels of the toxicant without warning. Wills [145] has noted that animals given atropine before respiratory exposure to sarin vapor were somewhat more susceptible to the poison than controls given no antidote. The ineffectiveness of atropine in this situation was interpreted as being due to its action to prevent the bronchoconstriction which otherwise occurs to some degree after inhalation of nerve gas vapor (see "Symptoms of Organic Phosphorus Poisoning"), therefore resulting in greater inhalation and absorption from the respiratory tract. Patients who are sick enough to receive atropine should be hospitalized and kept under careful observation for 24 hours; in some instances, a marked improvement has been noted after atropine followed later by deterioration of the patient's condition, requiring further treatment promptly.[71]

Ocular symptoms produced by local absorption do not respond to the systemic administration of atropine but are relieved by the local administration of 2% homatropine or, if severe, by the local administration of 0.5% or 1% atropine.[157] Parenteral atropine may relieve miosis, but the effect is both irregular and transient. If local ocular effects are present, the size of the pupils obviously cannot be used as an indicator of systemic poisoning or as a gauge for atropine dosage.

If convulsions interfere with artificial respiration and are not relieved by intravenous atropine, the patient may be given trimethadione (Tridione), a barbiturate, or ether. Trimethadione may be given intravenously or intramuscularly, in doses of 1 gm., repeated if necessary. Morphine should not be given. The action of succinyl choline (Suxamethonium) increases the effect of the anticholinesterase agents, and thus the use of this relaxant is contraindicated (see "Chemical and Physiologic Properties of Oximes").

Results of treatment using transfusion with normal blood or even intravenous infusion of purified cholinesterase have, in general, been disappointing.[14] It is doubtful whether these materials provide any active enzyme to important sites in the nervous tissue. Their action in combining with any circulating poison is too little and too late to be of value in reversing the pathology associated with poisoning.

It is important to understand that, while atropine is effective as an antidote, it has no effect on the fundamental biochemical lesion involved in poisoning by anticholinesterase compounds. Atropine merely serves to block certain actions of the excess acetylcholine already accumulated. Atropine has no action on the nicotonic effects of acetylcholine, including the neuromuscular block that leads to muscle weakness, and may finally end in death due to paralysis of the respiratory muscles.[160] Although atropine specifically blocks the muscarinic actions of acetylcholine, it is less effective in blocking certain muscarinic actions (such as those on the intestine and urinary bladder) than others (such as cardiac slowing and sialorrhea). The effects of anticholinesterases on the central nervous system are reversed by atropine, and the increased electrical activity of the brain, as shown by the EEG, is returned to normal.[157]

Tests with various synthetic parasympatholytic agents, including *l*-*N*-butylscopolammonium bromide (Buscopan),[37] have not shown any of these compounds to be significantly more effective than atropine in antidotal action.

Development of Specific Antidotes

Numerous investigators have sought to find agents that would interfere in the combination of the organic phosphorus compound with cholinesterase or hasten the destruction of the inactive phosphorylated enzyme complex. The development of specific antidotes of this type has been reviewed by Davies and Green.[23] The role of oximes in the treatment of anticholinesterase poisoning has also been reviewed by Wills[145] and by Verhulst and Page.[161]

Probably the usage of physostigmine (eserine) and neostigmine[26,70] represents the earliest record of such attempts. It was found that, instead of an expected summation or potentiation of effect occurring between DFP and physostigmine or neostigmine, prior administration of one of the latter agents protected rats against lethal doses of DFP. It was postulated that this antidotal effect was due to the formation between the physostigmine or neostigmine and cholinesterase of an unstable bond which served to protect the active site on the enzyme from attack by the irreversible inhibitor, DFP. The effectiveness of these particular agents is strictly prophylactic, and they have no antidotal effect when given after the poison. An additive rather than an antagonistic action was noted when physostigmine was given after DFP[97] or parathion.[129]

Somewhat later, Wilson[148,149] reported that choline and hydroxylamine were effective in reversing the combination between cholinesterase and certain organic phosphorus inhibitors. Wagner-Jauregg and his co-workers[146] used certain metal salts and chelates to reactivate DFP-inhibited enzyme.

The most promising development, however, is that associated with the hydroxamic acids and oximes.[150] As pointed out above, it has been shown that the organic phosphorus compounds inactivate cholinesterase by forming a firm bond with the enzyme. This combination is commonly spoken of as "irreversible" in contrast to the combination of cholinesterase with physostigmine or neostigmine, which can easily be reversed by dilution or dialysis. Actually, "irreversible" must be regarded here as a relative term. In fact, TEPP-inactivated enzyme spontaneously recovers some of its activity in vitro if allowed to stand in water for a sufficiently long time. It is more difficult to reactivate DFP-inhibited enzyme.

It would be expected that reagents (such as esters or acids) capable of making a nucleophilic attack on the phosphorus atom of the inhibitor-enzyme complex could regenerate the enzyme. Theory would further predict that presence within the reactivating molecule of a cationic structure, such as the ammonium radical, would facilitate this reaction by acting, as does the nitrogen atom in acetylcholine, to properly position the molecule for its reaction with the enzyme. The hydroxamic acids and oximes fulfill these requirements imposed by theory by having both nucleophilic and cationic ammonium groupings in the molecule.

The mechanism of action of oximes in regenerating inhibited cholinesterase is thought to consist of an initial direct combination between the organic phosphorus-inhibited enzyme and the hydroxamic acid, followed by a reaction in which the organic phosphorus moiety is split off and hydrolyzed. The hydroxamic acid residue undergoes a further reaction to regenerate the active enzyme. The important thing from a practical standpoint is that the hydroxamic acid has a greater affinity for the phosphorus moiety than does the enzyme.

Epstein and Freeman[48] have reported the results of a study of the toxicity and prophylactic and therapeutic efficiency of a series of 15 hydroxamic acids in nerve-gas poisoning in mice. Other workers at the Army Chemical Center[39] have made and tested 22 oximes in this regard. O'Leary et al.[118] have tested a number of oximes both singly and in various combinations.

Of the hydroxamates and oximes tested by various workers, the outstanding compounds are 2-pyridine aldoxime methiodide (2-PAM iodide) and related salts. These compounds combine the features of low mammalian toxicity and good prophylactic and therapeutic efficiency against organic phosphorus poisoning.

Details regarding usage of oximes, and particularly of 2-PAM salts, in the treatment of anticholinesterase poisoning are considered in succeeding sections of this review.

2-Pyridine aldoxime methyl methanesulfonate (P2S), which is the methane sulfonate salt corresponding to 2-PAM, has recently been shown to be just as effective therapeutically as 2-PAM iodide[35] and to possess the advantage of greater water solubility.[34] The methochloride salt of 2-pyridine aldoxime (2-PAM chloride) is more stable and more water soluble than 2-PAM iodide. The methochloride salt is now commercially available in the United States as an investigational drug under the trade name Protopam.* The lactate salt has been studied by O'Leary et al.[118]

In comparing the effects of these various salts of 2-pyridine aldoxime, it is necessary to take into account the variation in the amount of actual free base in the dosage administered. For example, 1.0 gm. of 2-PAM chloride is approximately equal to 1.5 gm. of 2-PAM iodide with regard to content of the active free-base, 2-pyridine aldoxime.

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* From Campbell Pharmaceuticals, Inc., 121 E. 24th St., New York 10. (The use of trade names is for identification purposes only and does not constitute endorsement by the Public Health Service.)

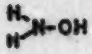
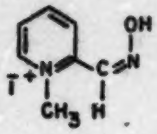
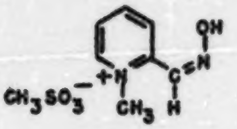
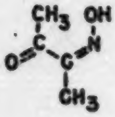
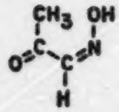
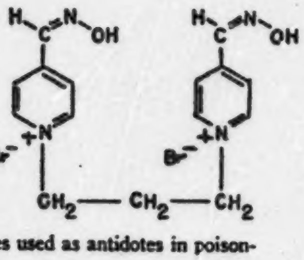
British workers [8,28,129] have proposed monoisonitroacetone (MINA) and diacetylmonoxime (DAM) as being superior to 2-PAM in combating organic phosphorus poisoning. DAM is less toxic than 2-PAM.[6] These oximes reactivate sarin-inhibited cholinesterase in vitro and are effective antidotes for sarin poisoning in rats. Hobbiger and his co-workers [79-81] have tested a series of 18 pyridinium oximes against TEPP and DFP both in vitro and in vivo. They reported that certain monoximes and dioximes of polymethylenebispyridinium compounds were from 15 to 52 times as potent as 2-PAM. Poziomek et al.[119] have also synthesized a number of compounds in this series. The outstanding antidotal compound from this group is 1,1′-trimethylene-bis(4-formyl-pyridinium bromide) dioxime (TMB-4).[18,52,79-81] These other oximes have not been studied so thoroughly as the salts of 2-PAM.

Another type of specific antidote which should be mentioned includes compounds which increase the hydrolytic activity of cholinesterase. Among the compounds which have been shown in vitro to possess this sort of activity are tryptamine,[50] certain analgesics,[53] and other compounds.[55,72,139] Most of this work has been carried out with plasma cholinesterase, although some studies using the specific enzyme have been done.[110,134,137] There is, at present, little or no indication that these agents would be of practical value in the treatment of poisoning.

Ball and his co-workers [10] found that rats which had been given a large but sublethal dosage of aldrin were able to withstand amounts of parathion that would ordinarily be lethal. This protective effect of aldrin was accompanied by an increased plasma cholinesterase level. Similar results have been noted with other chlorinated hydrocarbon insecticides, including chlordane, dieldrin, DDT, heptachlor, and the α and γ isomer (lindane) of benzene hexachloride (BHC).[29,115] Other known hepatotoxic agents (such as carbon tetrachloride) have a similar action. The transitory rise in serum esterase apparently is a temporary consequence of the tissue damage occurring in the liver. Since the protective effect has been noted for a direct inhibitor (TEPP) as well as for compounds which must undergo biotransformation in order to become toxic, a decreased activating ability of the liver would not seem to be the mechanism of action. The most likely possibilities appear to be an increased level of phosphorylatable enzyme which competes with the true cholinesterase for the inhibitor, or an increased level of hydrolyzing enzyme which aids in the degradation of the anticholinesterase. O'Brien [116] considers the latter possibility to be more likely.

Many of the organic phosphorus compounds (including parathion, the other phosphorothioates, and some phosphoroamidates) become potent anticholinesterase agents only after bioactivation by the liver.[116] It has proved possible to protect animals from compounds of this type by blocking the activating enzymes present in the liver. Thus, Davison [38] was able to protect mice from poisoning by octamethyl pyrophosphoramide (OMPA) but not by parathion through prior administration of the liver microsome inhibitor SKF 525A (β-diethylaminoethyl diphenylpropylacetate hydrochloride). O'Brien and Davison [117] studied other liver microsome inhibitors, but found SKF 525A to be the best of 12 agents tested. They confirmed the failure of SKF 525A to protect against parathion but found that it antagonized Guthion insecticide poisoning. The lack of effectiveness of SKF 525A against parathion poisoning has not been satisfactorily explained. The failure does not seem to represent a general ineffectiveness against phosphorothionates, since the LD_{50} of Guthion was increased.

In connection with this effect on the enzyme system which activates the organic phosphorus insecticides, it is interesting to note that compounds are also known which inhibit the enzyme systems that detoxify the organic phosphorus compounds. Among the compounds producing this effect are certain of the organic phosphates themselves. Thus, when given in combination, the resultant toxic action of certain of the organic phosphorus compounds is greater than would be expected on the basis of simple additive effect.[89] This potentiation was first reported for malathion plus ethyl-*p*-nitrophenyl thionobenzene phosphonate (EPN),[90] while the maximum synergistic effect (88 to 134 times) noted has been observed with malathion plus triorthotolyl phosphate TOTP.[100] The mechanism by which potentiation occurs has been elucidated and appears to involve the interference of one compound with the metabolism of the

Chemical Name	Common Name	Structural Formula
Hydroxyl amine	——	
Pyridine-2-aldoxime methiodide	2-PAM or 2-PAM iodide	
Pyridine-2-aldoxime methochloride	2-PAM chloride or Protopam	Same as above except chlorine salt
Pyridine-2-aldoxime methyl methanesulfonate	P2S	
Diacetylmonoxime	DAM	
Monoisonitrosoacetone	MINA	
1,1'-trimethylene bis(4-formyl-pyridinium bromide) dioxime	TMB-4 or TMB-4 dibromide	

Chemical name, common name, and structural formula of oximes used as antidotes in poisoning by anticholinesterases.

Toxicity of Oximes to Experimental Animals

Compound	Species	Sex	Route	LD$_{50}$ mg/kg	Reference
2-PAM iodide	Mouse	Mixed	Intraperitoneal	136± 6	92
2-PAM iodide	Mouse	Mixed	Intraperitoneal	223±10	103
2-PAM iodide	Mouse	Unstated	Intraperitoneal	200±22	109
2-PAM iodide	Mouse	Unstated	Subcutaneous	160±15	103
2-PAM iodide	Mouse	Unstated	Intravenous	145± 8	118
2-PAM sulfonate	Mouse	Unstated	Intravenous	116±13	118
2-PAM chloride	Mouse	Unstated	Intravenous	115± 3	118
2-PAM chloride	Rabbit	Mixed	Intravenous	96	118
TMB-4 dibromide	Mouse	Unstated	Intravenous	58± 8	118
TMB-4 dichloride	Mouse	Unstated	Intravenous	87± 1	118
TMB-4 dichloride	Rabbit	Mixed	Intravenous	44	118

second.[27,28,100] Seume and O'Brien[123] have reported that inhibition of carboxyesterase and carboxyamidase is important in potentiation of these compounds.

A potentiating effect between certain of the organic phosphorus insecticides and some phenothiazine-derived tranquilizers has been found to occur in rats[64] and has been suspected to occur in man.[3]

It has been noted that the signs and symptoms of parathion poisoning were intensified and prolonged in a patient who was given succinylcholine for control of convulsions.[120]

Chemical and Physiologic Properties of Oximes

The chemical structure of 2-PAM iodide is shown in the Figure. The similarity of the aliphatic portion of this molecule to hydroxylamine is apparent. Pure 2-PAM iodide is a yellow, crystalline solid which decomposes at 219 C. It is soluble in water up to about 5% and practically insoluble in ethyl alcohol. The methanesulfonate salt (P2S) is soluble in water up to about 40%.[34] The chloride is soluble in water to the extent of 1.0 gm. in less than 1.0 ml.

The toxicity to laboratory animals of various oximes as determined by a number of different investigators is summarized in the Table. It can be seen that the estimate of the intraperitoneal LD$_{50}$ of 2-PAM iodide to mice ranges from 136 to 223 mg. per kilogram, and the intravenous LD$_{50}$ ranges from 44 to 145 mg. per kilogram for mice and rabbits. The toxicity of the compound, while important, is not especially great. When lethal doses are given, death usually occurs within 10 to 20 minutes and is due to respiratory failure. Generalized clonic convulsions and gasping respiratory movements are seen. Generalized skeletal muscle tremors continue for a few minutes after cessation of respiration. The similarity of this symptom picture to that seen in organic phosphorus poisoning leads one to suspect that the mechanism of toxic action of 2-PAM may involve its observed capacity to inhibit cholinesterase in excess dosages (see further details on page 34).

In man, side-effects from the oximes 2-PAM iodide, 2-PAM chloride, and diacetylmonoxime (DAM) have been minimal in normal subjects and practically nonexistent in people who were poisoned.[68,69,87] Rapid intravenous injection of 2-PAM iodide has produced transient mild weakness, diplopia, blurred vision, dizziness, impairment of accommodation, and occasionally headache, nausea, and tachycardia.[87] A few patients given 2-PAM iodide have complained of a bitter taste that no doubt resulted from the iodine moiety of the molecule; 2-PAM iodide has also produced an allergic rhinitis and a feeling of fatigue in the jaws.[113]

Intravenous injection of DAM has caused a burning sensation at the site of injection radiating up the vein, giddiness, drowsiness, a sensation of warmth and tingling in the abdomen and chest, tachycardia, mild postural hypotension,[68] and occasionally bitter taste, paresthesias and decreased position sense in the extremities, decreased sweating, transient loss of consciousness, clonic movements of the head, and decreased amplitude of the electroencephalogram and of the T-wave segment of the electrocardiogram.[87]

Many of the oximes liberate cyanide in the body and also on long standing in vitro.[6,10] When there is doubt concerning the integrity of a 2-PAM preparation, particularly solutions of old or uncertain age, a simple test for free cyanide should be carried out.

It should be noted that 2-PAM salts have at least 3 actions which may be of importance in their effect on animals poisoned by organic phosphorus compounds. These actions are:

1. Reactivation of inhibited cholinesterase.
2. Reaction with and inactivation of the organic phosphorus molecule.
3. Inhibition of cholinesterase, especially in excess dosage.

The reactivation of inhibited cholinesterase is the dominant effect of 2-PAM in poisoned animals. In vitro, 2-PAM is a very powerful reactivator. At a concentration of 10^{-6}M., 2-PAM iodide reactivates as much as 80% of alkyl phosphate-inhibited enzyme within 1 minute.[154,21,23] The diisopropyl phosphorylated enzyme is reactivated more slowly and less completely than the diethyl substituted cholinesterase.[77] In a comparative in vitro study of 2-PAM iodide, DAM, and monoisonitrosoacetone (MINA), Cohen and Wiersinga[24] found that 2-PAM iodide was the most efficient reactivator, followed closely by MINA. The reactivation by DAM was slow when compared with those of the 2 other oximes. In a later paper, Cohen and Wiersinga[25] theorized that the antidotal efficacy of DAM was primarily due to its ability to inactivate the organic phosphorus molecule rather than its capacity to regenerate inhibited cholinesterase. Rajapurkar and Panjwani[124] suggested that DAM had an antiacetylcholine effect, like that of atropine. Their hypothesis was based on study of ciliary movement in the frog esophagus. This preparation has been shown to contain the acetylcholine-cholinesterase-choline acetylase system, but no nervous tissue. DAM produced slowing of the ciliary movement similar to that produced by atropine or curare. The action was the same in eserinized or normal cilia. Thus, apparently neither a central mechanism nor a reactivation of inhibited enzyme was responsible for the effect of DAM in this instance.

Kewitz[90] has shown that 2-PAM iodide reactivates the esterase activity of diaphragm in the living animal poisoned with paraoxon or DFP, but not OMPA. These results parallel those obtained in vitro with the same compounds and afford good evidence that the antidotal properties of 2-PAM depend upon reactivation of inhibited cholinesterase. Cohen and Wiersinga[26] reported that both MINA and 2-PAM iodide regenerated diaphragm cholinesterase in vivo in sarin-poisoned rats. Inhibited brain cholinesterase was reactivated by MINA only.

Some workers[7,78] have not been entirely satisfied with this explanation for the antidotal efficacy of 2-PAM, and, in support of their view, point out that the therapeutic efficacy and in vitro cholinesterase reactivating power of the oximes do not correlate very closely. One would do well to remember, however, that the other 2 actions of oximes (numbers 2 and 3 above) must also be considered and may explain the observed lack of correlation.

In contrast to the marked rise of enzyme activity in blood and muscle following the administration of 2-PAM, little or no effect has been noted on inhibited brain cholinesterase.[78,128,90,91,25] This failure of 2-PAM to reactivate brain cholinesterase has usually been ascribed to a supposed inability of the oxime to pass the blood-brain barrier. In earlier work, Koelle and Steiner[96] had shown that quaternary nitrogen compounds penetrate the blood-brain barrier with difficulty. However, even when administered intracisternally, P2S had no effect on the central actions of sarin. Holmstedt[84] considered that the observed failure of 2-PAM iodide to restore respiration depressed by anticholinesterase was due to its inability to penetrate the blood-brain barrier. Jager et al.[88] found low concentrations of 2-PAM iodide in the brain of 2 rabbits infused with this antidote. Wilson[152] has attempted to overcome this difficulty by using the fat-soluble dodecanoic acid analogue of 2-PAM with only partial success. In contrast to these results, Rosenberg[130] has noted in vivo reactivation of brain cholinesterase by 2-PAM iodide after paraoxon poisoning in the rabbit. Rosenberg considered that the differences between his results and those of others cited above might be due to species variation or to the use of different extraction procedures. Differences in dosage levels of 2-PAM may also have been a factor, since Rosenberg gave very high dosages (up to 250 mg. per kilogram).

The marked effectiveness of 2-PAM iodide, 2-PAM chloride, and TMB-4 in poisoning by Phospholine Iodide in mice is considered to be related to the fact that phospholine, in contrast to other organic phosphorus cholinesterase inhibitors, contains a quaternary nitrogen atom, and thus, like the quaternary oximes, fails to pass the blood-brain barrier.[99]

TMB-4 did not reactivate brain cholinesterase activity in rats poisoned with DFP or sarin.[51]

Jager et al.[88] were not able to demonstrate 2-PAM iodide in the cerebrospinal fluid in a man who received 44 mg. per kilogram of this oxime intravenously. However, rapid returns to consciousness following administration of 2-PAM iodide have been noted in patients poisoned with parathion.[99,49,121] This clearing of consciousness would seem to be an indication that the drug has access to the brain in man. The practical clinical result may be further evidence for species variation in the action of 2-PAM.

In contrast to the peripherally-acting quaternary oximes (2-PAM and TMB-4), the nonquaternary oximes have a predominantly central action.[146,19] DAM has been shown to penetrate the blood-brain barrier and effect some reactivation of brain cholinesterase.[88,43] However, DAM in large doses has produced coma in man.[87]

O'Leary et al.[118] have reported that atropine and a combination of a monoquaternary and a bisquaternary oxime were superior to atropine with either single type of oxime in dogs and rabbits poisoned with sarin or tabun. A 1:1 mixture of 2-PAM chloride and TMB-4 dichloride was the most effective combination tested. Edery and Schatzberg-Porath[44] have noted synergism between 2-PAM and DAM.

At any given dosage with any given inhibitor and reactivator, the extent of reactivation of cholinesterase activity that occurs depends upon how long the inhibitor and enzyme have been left in contact.[76] Hobbiger theorized that the phosphorylation of cholinesterase led initially to an unstable phosphorylated enzyme which gradually changed to an irreversibly phosphorylated enzyme. This phenomenon has been termed "aging" by O'Brien[116] and has been shown to occur in vivo[76] as well as in vitro.[131,82] Aging occurs rather slowly with methylphosphorylated cholinesterase, but more rapidly with diethyl- or diisopropyl-substituted enzyme.[32] These results indicate that the oximes might be less effective in regenerating cholinesterase inhibited during repeated exposure than that inactivated after a single exposure.

The use in vitro of a reactivator in the routine cholinesterase test has been proposed to allow determination of both inhibited and normal enzyme levels in a single blood sample from a patient in whom poisoning is suspected.[3] The occurrence of aging would seem to make achievement of this objective unlikely in connection with repeated exposure; but it should be possible, by use of a similar technique, to differentiate between an inhibition of cholinesterase due to a recent, acute exposure and a depression due to a previous or repeated exposure.

Direct reaction between 2-PAM and the organic phosphorus molecule has been shown to occur.[142,77] The importance of this reaction in either the prevention of dermal absorption or local effects of anticholinesterase compounds or both has been discussed by Summerson.[159] Kewitz, Wilson, and Nachmansohn[93] have, however, pointed out that it is unlikely that the direct reaction is an important factor in the antidotal action on systemic effects in vivo, since in vitro experiments have shown that this direct reaction is very slow at concentrations obtainable under physiological conditions. These authors note that even with a 10^{-3}M. concentration of 2-PAM iodide at 25 C, pH 7.8, only about

1% of diethyl *p*-nitrophenyl phosphate (Paraoxon, 10^{-4}M.) reacts per hour. Cohen and Wiersinga[25] found that both DAM and MINA detoxified sarin during in vitro incubation. No detoxication of sarin by 2-PAM iodide occurred. These authors suggested that DAM acts mainly by virtue of direct reaction with the toxicant, that 2-PAM acts primarily by reactivating inhibited enzyme, and that MINA shows both types of activity.

At sufficiently high dosages, 2-PAM iodide is capable of inhibiting both serum and erythrocyte cholinesterase in vitro.[82,77,88] Although the antidotal dosages which have generally been used are below the toxic level, there would seem to be, on the basis of studies reported by Loomis,[102] a definite possibility of lessened antidotal effect if greater than the optimal dose of 2-PAM were given. This author noted that dogs given an intravenous dose of 60μg. of sarin per kilogram required more than 240 minutes to recover their normal response to injected acetylcholine. Following intravenous administration of 10, 25, or 250 mg. per kilogram of 2-PAM iodide to normal dogs, <2, 3, and >120 minutes, respectively, were required for return of the normal acetylcholine response. The same dosage levels of 2-PAM iodide when given to sarin-poisoned dogs, produced return to normal acetylcholine response after 40 to 45, 80 to 100, and >120 minutes, respectively. These results indicate that 2-PAM iodide is a cholinesterase inhibitor, that 10 mg. per kilogram was the optimum antidotal dosage under the condition of the test and that larger doses were less effective.

Woodard[158] gave 50 mg. per kilogram of 2-PAM iodide to cattle intraperitoneally and intravenously. This dosage produced some restlessness and mild abdominal distress, but the signs were mild and of short duration. One sheep given 25 mg. per kilogram of 2-PAM iodide intravenously showed the same signs as the cattle, plus an increased respiratory rate and excessive salivation. No change in erythrocyte cholinesterase level was observed in any of these animals.

The cholinesterase inhibitory activity of 2-PAM has also been noted by Bergner and Wagley.[16] These workers reported that 2-PAM iodide in high concentration in vitro had a dual effect on motor end-plate cholinesterase previously inactivated by TEPP. The 2-PAM iodide reactivated the enzyme and, if allowed to remain in contact with the preparation, inhibited the reactivated enzyme.

It is important to note that in poisoned people given therapeutic doses, the inhibition of cholinesterase by 2-PAM is trivial, while the release of enzyme inhibited by the organic phosphorus compound is highly significant.

Kewitz et al.[93] have carried out experiments which show that 2-PAM does not have an atropine-like action. In studies on the blood pressure of eviscerated cats, the effect of acetylcholine was not modified by 2-PAM iodide, even when large doses were used; this effect was completely abolished by a small dose of atropine, however. After 100 mg. per kilogram of 2-PAM iodide, these authors reported a rise in blood pressure from 120 to 160 mm. Hg which lasted 15 to 20 minutes and was abolished by atropine. This effect from the very large dose of 2-PAM iodide was not further studied. Possibly, it was a reflection of the cholinesterase inhibitory effect of 2-PAM.

Both 2-PAM iodide and DAM, when injected intravenously in large doses, have a direct depressant action on the respiratory center.[156]

The duration of action of a single dose of 2-PAM has been studied by Kewitz et al.[93] Following intraperitoneal dosage, 2-PAM iodide reached its maximum effect in rats within 30 minutes and had declined to about 50% of maximum effectiveness at the end of the first hour. A further decline in activity took place during the second hour, but even at the end of 2 hours, some protective effect was still evident. Following intravenous dosing, 2-PAM iodide appears to be well dispersed in all body tissues with the exception of the brain.[83] These same authors found that 2-PAM iodide was rapidly excreted in man following intravenous dosage. The half-life was 0.9 hours. Excretion was primarily by the kidneys. In nephrectomized dogs and rats and in a patient with chronic nephritis and azotemia, excretion was retarded. In vitro, 2-PAM iodide was metabolized aerobically by rat liver slices.

P2S is also rapidly excreted in man following intravenous dosing.[136] Absorption of P2S after intramuscular or oral administration was not satisfactory.

DAM is excreted somewhat more slowly in man than is 2-PAM iodide.[85] The half-life for the former compound is 7.2 hours. Relatively little of the DAM could be recovered from the urine. Apparently, the liver is of major importance in the metabolism of DAM.[39,66]

Loomis [103] has tried 2-PAM in the treatment of local ocular effects of TEPP and sarin in rabbits. He found that 2-PAM iodide applied locally was very effective in reversing miosis induced by organic phosphorus compounds. The reversal was brought about by 2-PAM iodide in 2 to 3 hours; whereas, if allowed to remit spontaneously, it required from 24 to 48 hours or even longer. The most effective dosage form was a petrolatum-based ointment, containing 0.1% of 2-PAM iodide. In contrast to Loomis' results, Kewitz et al.[93] reported that local application of 2-PAM iodide had no effect on a rabbit's pupil constricted by placing diethyl *p*-nitrophenyl phosphate in the eye. However, intravenous injection of 50 mg. per kilogram of 2-PAM iodide brought the pupil back to normal size and restored reactivity to light within 5 minutes. The formulation in which 2-PAM iodide was used for local application was not stated. It may be that the lack of effect was due to use of a water solution of 2-PAM iodide rather than an oil-based preparation.

Application of 2-PAM iodide [82,147] and MINA [82] relieves the neuromuscular block caused by anticholinesterases. Grob and Johns [68] reported that DAM reversed the local neuromuscular block produced by anticholinesterases in man, but Edery [43] did not note this effect in the cat or the rat. In this connection, it would be of interest to study the effect of these oximes on the paralysis caused by certain organophosphorus compounds. Holmes and Robins [82] have stated that the reversal of neuromuscular block is due entirely to a reactivation of inhibited cholinesterase in the muscle. Wislicki [186] noted that DAM reduced direct and indirect muscular excitability to a marked extent, while with 2-PAM iodide these effects were slight and were obtained only after intra-arterial administration. Application of 2-PAM antagonizes the block caused by the competitive type of blocking agent, such as curare, and it intensifies the effect of suxamethonium. DAM has no definitive influence on the former but prolongs the action of the latter. Neostigmine, unlike atropine, antagonizes the depression of muscle excitability by DAM but has no marked effect on the action of 2-PAM.

Longo et al.[101] have reported that the threshold level of sarin necessary to produce a "grand mal" electroencephalographic pattern was increased about threefold by 2-PAM iodide. However, 2-PAM iodide had no effect on the early phase of desynchronization of the electroencephalogram.

Antidotal Efficacy of Oximes in Poisoned Experimental Animals

There are now in the published literature numerous reports giving results of the usage of 2-PAM salts and other oximes in laboratory animals poisoned with various organic phosphorus compounds. The compounds studied have included demeton,[130] DFP,[93,20,78,52] Diazinon,[131] dimefox,[131] dimethoate,[131] endothion,[54] Ethyl Guthion,[130] Guthion,[130] methyl demeton,[54] methyl parathion,[131] morphothion,[130] paraoxon,[92,93,17,78] parathion,[17,155,54,131] phencapton,[131] Phosdrin,[130] phosphamidon,[130,86,108] Phospholine Iodide,[99] sarin,† OMPA,[93] tabun, [20,153,52] TEPP,[78,155,35,52] and phorate.[130] Most workers have found that the combination of oxime and atropine is superior to either antidote used alone. However, Sanderson [130] did not note any increased benefit of an oxime (2-PAM iodide or P2S) plus atropine in comparison with atropine alone in rats given oral doses of 10 organic phosphorus and carbamate insecticides. With oral Guthion, Ethyl Guthion, demeton, and morphothion, 2-PAM iodide apparently reduced the beneficial effect of atropine. This author did note potentiation between atropine and oxime in some instances when the poison was given intraperitoneally. Sanderson and Edson [131] concluded that the relative effectiveness of oxime therapy with different organic phosphorus insecticides depends on the proportion of reversible cholinesterase inhibition present, as predicted on the basis of the structure and duration of action of the anticholinesterase compound.

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† References 102, 6, 147, 155, 35, 145, and 52.

The efficacy of 2-PAM in the treatment of poisoning varies from compound to compound, but it has shown some effectiveness in the case of all the materials listed above with the exception of dimefox, dimethoate, and OMPA. In this connection, it is interesting to note that Tammelin and Enander [128] reported synthesis of an organic phosphorus compound (cholinyl methylphosphonofluoridate) which caused an inhibition of cholinesterase that could not be reversed in vitro by 2-PAM iodide.

Woodard [158] has reported results of studies with 2-PAM iodide in cattle and sheep poisoned with organic phosphorus insecticides. Neither atropine nor 2-PAM iodide alone was a satisfactory antidote in these animals. A combination of 2-PAM iodide and atropine gave good results on cattle poisoned with parathion or Diazinon, but not on cattle poisoned with malathion or on sheep poisoned with parathion.

It appears likely that different oximes have some specificity not only for different organic phosphorus compounds but also for different species.

The effectiveness of oximes as antidotes has also been tested in poisoning by certain other cholinesterase inhibitors which are not organic phosphorus compounds. The use of 2-PAM iodide has been reported to be ineffective, if not detrimental, in the treatment of poisoning by the carbamate insecticide Sevin in rats and dogs.[22,130] On the other hand, poisoning in the rat by the carbamate insecticides Isolan and Dimetilan was benefited by 2-PAM iodide.[130] It has also been shown that 2-PAM iodide and DAM reverse the physiological effects of neostigmine (which is a substituted carbamate) and several of its derivatives.[68]

Application of 2-PAM in Poisoning in Man

The oxime 2-PAM has now been used in the treatment of poisoning in man in a number of instances.

Namba and Hiraki [114] have reported a series of 5 cases. All of these cases were classified by the authors as serious—indicating a potentially fatal outcome—if untreated. However, one of those patients was already asymptomatic at the time he received 2-PAM iodide. A second patient who had previously received 10 mg. of atropine showed no clinical improvement immediately after receiving 100 mg. of 2-PAM iodide intravenously. He did, however, improve about 3 hours later (6 hours after onset of symptoms). A third case was given ten 100-mg. intravenous doses of 2-PAM iodide during a period of 3.5 hours. By the end of this time, all symptoms had disappeared. A fourth patient became symptom-free after doses of 500 and 400 mg. of 2-PAM iodide, separated by 10 minutes. The fifth case received 1 gm. of 2-PAM iodide intravenously, which the authors considered to represent the ideal treatment. Within 21 minutes, all the symptoms in this fifth case, including muscle fasciculations, had disappeared.

In 3 of the 5 cases cited above, the erythrocyte cholinesterase level returned to normal in 10 minutes, 40 minutes, and 3.5 hours, respectively. In the fourth case the recovery of the red-cell enzyme was significantly more rapid than that of the untreated control. In the fifth case, it is not clear from the data presented that the red-cell enzyme level was ever significantly depressed. No immediate effect of 2-PAM iodide on plasma cholinesterase was evident, but in all treated cases studied, this enzyme returned to a normal level more rapidly than did the untreated control.

It should be especially noted that the recovery in 4 out of 5 of these human cases was after 2-PAM iodide alone. Only 1 of the patients received a combination of 2-PAM iodide and atropine. The work with experimental animals cited above would lead one to expect that a combination of both drugs would be more effective than either alone.

In a later paper, Namba[112] reported the results of the use of 2-PAM iodide in an additional 26 cases of accidental parathion poisoning and in 2 cases of attempted suicide by ingestion of parathion. The accidental poisonings were treated with 1 to 2 gm. of 2-PAM iodide intravenously. Marked improvement was noted within 30 minutes. Pallor was the only symptom remaining in 5 cases, miosis in 3 cases, and difficulty of speech and tachypnea in 2 cases. These remaining symptoms had cleared within 1 hour after treatment. Complete recovery of consciousness occurred in 5 cases. Symptoms such as nausea, headache, dizziness, and numbness of extremities did not respond as well to 2-PAM as did the others just mentioned. Some of these effects persisted for as long as 1 week. To be effective in cases of ingestion of parathion, it was necessary to give very large doses of 2-PAM. Continuous intravenous infusion of 2-PAM at the rate of 0.5 gm. per hour is preferred by Dr. Namba. One patient received a total dose of 40.5 gm. of 2-PAM iodide, recovering without apparent ill effect. Namba[112] mentioned exploratory study of the use of 2-PAM for the prophylaxis of parathion poisoning.

Funckes[62] has reported the successful treatment with 2-PAM iodide and atropine of a severe case of occupational parathion poisoning. This patient was given 1 gm. of 2-PAM iodide intravenously about 1 hour after illness began, and he improved dramatically within 10 minutes. Although blood cholinesterase levels prior to administration of 2-PAM iodide were not determined, the enzyme activities after the drug was given indicated reactivation of erythrocyte cholinesterase (0.32 Δ pH per hour) as measured by the method of Michel[107] and expressed in terms of change of pH per hour; there was little or no effect on plasma enzyme (0.04 Δ pH per hour).

Quinby and Clappison[121] used 2-PAM iodide in the treatment of a 2-year-old child who ate dirt contaminated with parathion. The child was given an intravenous dose of 250 mg. of 2-PAM iodide following repeated doses of atropine. The critically ill, unconscious child returned to an almost normal state in less than 20 minutes after the oxime dosage was given. The levels of both plasma and erythrocyte cholinesterase were near 0 before 2-PAM, but 20 minutes after its administration, the erythrocyte cholinesterase level had returned to within the range of normal. The reactivation of plasma enzyme level was much slower, the normal level being reached between the third and nineteenth days. *p*-Nitrophenol was excreted in the urine quite rapidly, clearing in about 30 hours from ingestion.

Schvartsman and his colleagues[132] treated a child who had ingested a commercial solution of parathion. The treatment included milk, atropine, stropanthin, and 300 mg. of 2-PAM sulfonate. The child regained consciousness and began to improve after treatment; he was discharged from the hospital after 3 days.

Karlog et al.[86] have reported the treatment with atropine and 2-PAM iodide of a patient who attempted suicide by taking an estimated 1.75 gm. of parathion by mouth. This patient was given very large doses of atropine beginning soon after poisoning. His respiration ceased, and he was given artificial respiration for 5 hours after poisoning. He was not given any 2-PAM until 2.5 days after poisoning. During the next 12 hours he was given 3.5 gm. of the antidote. Each dose of 2-PAM iodide produced transitory rises in plasma and erythrocyte cholinesterase activity which persisted for only a few hours, but the patient eventually recovered.

Rosen[125] treated with 2-PAM iodide a young man who was poisoned by accidentally spilling a gallon bottle of parathion concentrate on his hands. This patient was given adequate doses of atropine during the first 18 hours after poisoning, and by the end of this time he was symptom-free except for persisting muscular weakness. After 2-PAM was administered, prompt improvement in muscle strength was noted.

One of the most severe poisoning cases treated with 2-PAM is that reported by Imo.[86] A young girl drank parathion with suicidal intent. She was found unconscious, in convulsions, foaming at the mouth, and unreactive even to the strongest stimulus. She had almost no pulse and was cyanotic and areflectic. Atropine, artificial respiration, Stereofundin, and nikethamide (Coramine) were given, and spontaneous respiration was restored. About 9 hours later, 500 mg. of 2-PAM iodide was given. Within 1 hour, the patient regained color, became completely conscious, and her pupillary reflexes returned to normal. Further recovery was uneventful.

Other instances of parathion poisoning treated with 2-PAM include a series of 10 cases (of which 6 were attempted suicide) reported by Erdmann.[47] Three of these patients recovered, 3 died, and in 4 cases the outcome is not indicated.

Erdmann and Latki[48] have used 2-PAM iodide in the treatment of a case of poisoning from inhalation of DFP. The administration of atropine and 2-PAM iodide (2 doses of 500 mg. each, orally, 2 and 5 hours after exposure) produced prompt remission of symptoms with the exception of miosis, which persisted for 8 to 10 days and led to a severe conjunctivitis. Some increase in erythrocyte cholinesterase activity was noted after 2-PAM, but the enzyme level did not return to normal until about 30 days later.

In 2 cases of moderately severe 2-carbomethoxy-1-methyl-vinyldimethyl phosphate (Phosdrin) poisoning, Funckes[63] noted a less dramatic clinical response to 2-PAM iodide than in the case of parathion poisoning. Both of the 2-PAM-treated Phosdrin cases continued to show symptoms of poisoning for several hours after receiving the antidote. However, the erythrocyte cholinesterase level was significantly increased, and the plasma enzyme was regenerated to a lesser degree after 2-PAM therapy.

A formulating plant worker studied by Quinby and Congdon[122] became poisoned while working with parathion. He had a very low blood cholinesterase level (plasma 0.10 and erythrocytes 0.08 Δ pH per hour) during his acute illness and was given atropine therapy. His gastrointestinal complaints, particularly anorexia and nausea, as well as weakness, headache, and malaise, persisted. He continued to work in the plant, although presumably he was removed from direct contact with insecticides. However, his urinary *p*-nitrophenol excretion indicated continued exposure to parathion. Because of these continuing symptoms, and in an attempt to raise his blood cholinesterase level sufficiently to permit his safe return to his regular work, this man was given 1 gm. of 2-PAM chloride 6 days after his acute illness. The antidote had little or no effect on his blood cholinesterase level, but he felt much better the next morning.

It is apparent from brief case reports that the use of 2-PAM in combination with atropine is considered effective and is routine in the treatment of poisoning by organic phosphorus insecticides in Israel.[2]

Suggestions for Treatment

The following suggestions are provided as a guide to the physician who may be called upon to treat persons poisoned by anticholinesterase compounds:

I. In very severe cases, the order of treatment should be as follows:
  (1) ARTIFICIAL RESPIRATION, preferably by mechanical means.
  (2) ATROPINE, 2 to 4 mg. (1/30 to 1/15 grain) intravenously as soon as cyanosis is overcome. Repeat at 5- to 10-minute intervals until signs of atropinization appear (dry, flushed skin and tachycardia as high as 140 per minute).
  (3) 2-PAM, 1 gm. *slowly*, intravenously.
  (4) DECONTAMINATION of the skin, stomach, and eyes as indicated.
  (5) SYMPTOMATIC TREATMENT.

II. In the more usual case, proceed as follows:
  (1) ATROPINE, 1 to 2 mg. (1/60 to 1/30 grain), if symptoms appear. If excessive secretions occur, keep the patient fully atropinized. Give atropine sulfate every hour up to 25 to 50 mg. in a day.

(2) **DECONTAMINATION** of the skin, stomach, and eyes as indicated.

(3) 2-PAM, 1 gm. slowly, intravenously if the patient fails to respond satisfactorily to atropine.

(4) SYMPTOMATIC TREATMENT.

It will be noted that this recommended dosage of atropine is greater than that conventionally employed for other purposes but is within safe limits. People poisoned by anticholinesterase organic phosphorus compounds have an increased tolerance for atropine. For additional discussion of atropine usage, see "Use of Atropine and Other Nonspecific Antidotes," above.

Although different oximes offer somewhat different therapeutic possibilities, 2-PAM appears to be the most generally useful oxime now available. There appears to be no essential difference in the effects of the different salts of 2-PAM, and 2-PAM chloride does have the advantage of greater solubility and lack of iodine taste as compared to 2-PAM iodide, which used to be more easily available. A dosage of 10 to 20 mg. per kilogram has proved a safe but generally effective level. As a round figure, 1 gm. should be an average adult dose. Intravenous administration is recommended to obtain rapidity of action. Since 2-PAM is excreted rapidly, repeated doses may be necessary, particularly in cases involving parathion or other compounds that must undergo bioactivation before becoming toxic. It is important to note, however, that the only known signs or symptoms of overdosage with 2-PAM are identical to the manifestations of poisoning with anticholinesterases. 2-PAM should be administered in conjunction with adequate doses of atropine. Other supportive treatment, including particularly artificial respiration, should also be carried out as required.

The value of 2-PAM is most thoroughly established in connection with the treatment of poisoning by parathion. When used in cases of poisoning by other organic phosphorus insecticides, it should be with the awareness that it may be somewhat less effective. In fact, 2-PAM appears to be ineffective in treating poisoning due to OMPA. Until 2-PAM is freely available for prescription it is recommended that the limited supply not be drawn upon in cases of mild poisoning or in cases that show a completely satisfactory response to atropine.

Consideration should be given to local application of 2-PAM to the skin to reduce dermal absorption in exposed persons, and to the eyes to reverse organic-phosphorus-induced miosis. The use of 2-PAM for the prevention rather than the treatment of systemic poisoning has been proposed. These procedures seem to be safe on the basis of limited studies with experimental animals, although their effectiveness has not been proved. These procedures are strictly exploratory, while the value of 2-PAM for treatment of systemic poisoning, especially by parathion, is established.

Never give morphine, theophylline, or theophylline-ethylene-diamine (aminophylline) to a patient poisoned with an anticholinesterase agent. Do not give atropine to a cyanotic patient; give artificial respiration first, and then give atropine. Large amounts of intravenous fluids are generally contraindicated because of excessive fluid in the respiratory tract. Tranquilizers should be used with great caution; they are seldom indicated at all. Succinyl choline should not be given.

If pulmonary secretions have accumulated before atropine has become effective they should be removed by suction and a catheter. If the stomach is distended, empty it with a Levine tube.

If the patient has not yet shown symptoms or they have been allayed by treatment, he must be completely and quickly decontaminated. Remove the patient's clothing and, with due regard for his condition at the moment, bathe him thoroughly. Remove any visible insecticide gently with a generous amount of soap and water or other detergent if available. Avoid abrasion. When the skin appears clear, bathe or swab with ethyl alcohol. Parathion and many of the other organic phosphorus insecticides are very much more soluble in alcohol than in water, and significant amounts can be washed from skin that has been scrubbed several times with soap and water.

If there is any suspicion that the poison has been ingested or inhaled and if the patient is still responsive, induce vomiting, give some neutral material such as milk or water, and induce vomiting again. The reason for mentioning inhaled material is, of course, that a large portion of it is deposited in the upper respiratory tract and subsequently carried to the pharynx and swallowed. Nausea may be anticipated on the basis of the systemic action of these compounds, but if vomiting is not profuse, gastric lavage may be used. Experiments have indicated that vomiting induced immediately or even 1.5 hr. after ingestion is more effective than gastric lavage in removing poison.

It must be kept in mind how little parathion is necessary to produce poisoning by the oral route. Repeated dosage at the rate of 7.2 mg. per man per day led to moderate reduction of blood cholinesterase activity.[44a] A dose of 25 to 50 mg. was fatal to a child,[132a] and a dose of 120 mg. was fatal to an adult.[17a]

Atropine does not protect against muscular weakness. The usual mechanism of death appears to be respiratory failure. The use of an oxygen tent or even the use of oxygen under slight positive pressure is advisable and should be started early. Watch the patient constantly, since the need of artificial respiration may appear suddenly. Equipment for oxygen therapy and for artificial respiration should be placed at the patient's bedside in readiness while the patient is on his way to the hospital. Cyanosis should be prevented by the most suitable means, since continued anoxia aggravates the depression of the respiratory center caused by the poison directly. Complete recovery may occur even after many hours of artificial respiration have been necessary.

If there is any reason to think that the eyes may have been contaminated, irrigate them with isotonic saline solution or water. The absorption of some of the organic phosphorus insecticides by the eye is remarkably rapid.

The acute emergency lasts 24 to 48 hours, and the patient must be watched continuously during that time. Favorable response to one or more doses of atropine does not guarantee against sudden and fatal relapse. Medication must be continued during the entire emergency. Any person who is ill enough to receive a single dose of atropine should remain under medical observation for 24 hours, because the atropine may produce only a temporary relief of symptoms in what may prove to be a serious case of poisoning. Atropine should never be administered for preventive purposes to persons who have not become sick.

Miosis and headache may persist after recovery from poisoning by organic phosphorus insecticides is otherwise largely complete. In some cases, the systemic administration of atropine is followed by partial or temporary dilatation of the pupils. Miosis responds more dependably to 2-PAM. If further systemic treatment is not necessary, the miosis and associated headache will respond to the instillation of 0.5% to 1% atropine solution or 0.5% atropine ointment into the eyes.

Following exposure heavy enough to produce symptoms, further organic phosphorus insecticide exposure of any sort should be avoided. The patient may remain susceptible to relatively small exposures to the same or any other organic phosphorus compound until regeneration of cholinesterase is nearly complete.

Prevention of Poisoning

The most important single factor in the prevention of poisoning is a knowledge of the hazards involved in handling the various anticholinesterase compounds. It is essential that the facts about the toxicity of these materials be learned through research and then that these facts be made known to all concerned, including the formulating plant worker, the commercial sprayman, the farmer, the householder (who may purchase these insecticides for use in his house or garden and, if not adequately warned, may leave the toxic materials accessible to children or other irresponsible persons), and the physician who may be called upon to treat poisoning.

In persons occupationally exposed, poisoning can best be prevented by constant, thoughtful care and by use, where indicated, of safety devices such as protective clothing, respirators, masks, air-conditioned cabs, or special factory ventilation.

Prevention can also be approached by observing trends in serial blood cholinesterase determinations on persons constantly exposed. The interpretation of individual values in asymptomatic persons is difficult. It is clear, however, that a single very low enzyme value for one worker or a low average value for a group of exposed workers is an indication of the need for improved personal care or better mechanical protection or both. In a similar way, prevention of poisoning may be helped by proper evaluation of the amount of urinary excretion of biotransformation products.

Direct methods are also available to measure the exposure which spray operators have to pesticides during their usual conditions of work.[43]

Severe poisoning may at times be prevented by the immediate recognition and correction of contamination. Prompt, effective medical treatment may prevent a mild poisoning case from developing into a serious one.

Comment and Summary

Poisoning due to organic phosphorus compounds may be encountered in relation to their use as insecticides; as chemical warfare agents; or as drugs for the treatment of abdominal distention, glaucoma, or myasthenia gravis. The principal, if not the only, pharmacologic action of the organic phosphorus insecticides is inhibition of the enzyme cholinesterase. The inactivity of this enzyme results in an accumulation of unhydrolyzed acetylcholine and the appearance of signs and symptoms referable to overstimulation of the parasympathetic nervous system. The drug of choice in the treatment of organophosphorus poisoning has been atropine. Atropine therapy produces relief of certain symptoms based on blocking the action of excess acetylcholine, but it has no effect on the basic biochemical pathology involved in anticholinesterase poisoning. With regard to symptoms, atropine is effective in treating those effects referable to the central and muscarinic action of acetylcholine but has no effect on the nicotinic action.

More recently a series of antidotes has been developed that are able to reverse the combination between the cholinesterase molecule and the inhibitor. These compounds are oximes and include salts of, for example, 2-PAM (2-pyridine aldoxime methiodide), DAM (diacetylmonoxime), MINA (monoisonitrosoacetone) and TMB-4 (1,1′-trimethylene bis(4-formyl-pyridinium bromide) dioxime). Since the oximes and atropine have different sites of action in the mammalian body, it is preferable to use these 2 agents in combination and thus take advantage of the antidotal effects at both points.

The most widely used of the oximes in treating poisoning is 2-PAM. This compound has at least 3 actions that may be of importance in the treatment of poisoning by an organic phosphorus compound. These actions are: (1) reactivation of inhibited cholinesterase, (2) reaction with and inactivation of the organic phosphorus molecule, and (3) inhibition of cholinesterase. Atropine has none of these actions. The dominant effect of therapeutic doses of 2-PAM in poisoned animals is reactivation of inhibited cholinesterase. Application of 2-PAM reactivates inhibited cholinesterase both in vitro and in vivo, except that it has little or no effect on the enzyme in the brain. The nonquaternary oximes (such as DAM and MINA) are able to penetrate the blood-brain barrier. With any given inhibitor and reactivator, the extent of reactivation of cholinesterase activity depends upon how long the inhibitor and enzyme have been in contact.

Although the value of 2-PAM in the treatment of organic phosphorus poisoning was discovered in the United States and procedures for its use were developed here, and although this antidote is now widely available for use by physicians in a number of other countries (England, Israel, Japan, and New Zealand), it can be purchased in this country only by qualified physicians for clinical investigational use. It is unfortunate that no firm has seen fit to market 2-PAM commercially for prescription use in the United States.

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A RECIPROCAL RELATIONSHIP BETWEEN THE INDUCTION OF δ-AMINOLEVULINIC ACID SYNTHETASE AND DRUG METABOLISM PRODUCED BY *m*-DICHLOROBENZENE

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Abstract—The daily administration of large doses of *m*-dichlorobenzene (*m*-DCB) causes experimental hepatic porphyria in rats by induction of δ-aminolevulinic acid synthetase (ALA synthetase). However, smaller doses of *m*-DCB produce a biphasic stimulation of both urinary coproporphyrin excretion and liver ALA synthetase. The decline of ALA synthetase and urinary coproporphyrin despite continued daily dosage is associated with an increase in activity of the liver drug-metabolizing systems and decreasing serum *m*-DCB levels. The most probable explanation for the self-limiting action of *m*-DCB is stimulation by the drug of its own metabolism.

PHYSIOLOGIC control of heme synthesis appears to be exerted primarily through regulation of the initial and rate-limiting enzyme, δ-aminolevulinic acid synthetase (ALA synthetase).[1,2] A wide variety of drugs have been shown to induce this enzyme in the liver of experimental animals or cultured embryonic chick livers.[3,4] Heme, the end-product of this biosynthetic pathway, prevents the induction of ALA synthesis, possibly by feedback repression.[3,5–7]

Several investigators have noted that the rapid turnover of the microsomal cytochrome, P-450, accounts for at least 50 per cent of all the heme synthesized in the unstimulated liver of the rat[8] and mouse.[9] Cytochrome(s) P-450 plays a critical role as the terminal oxidase in the metabolism of drugs, steroids and heme.[10,11] Many drugs have been shown to induce the drug-metabolizing enzymes and concomitantly to increase microsomal cytochrome P-450.[10] Many of the compounds that stimulate drug metabolism (phenobarbital, chlorinated insecticides, griseofulvin, steroids) also induce ALA synthetase,[9] presumably to provide the heme needed for the increased synthesis of the microsomal cytochrome. Phenobarbital induction of ALA synthetase and the increase in cytochrome P-450 and microsomal protein are all abolished by the simultaneous administration of heme.[6] Hence, control of drug metabolism can be exerted by inhibiting ALA synthetase or other steps in the heme biosynthetic pathway.[12]

The following data suggest that induction of ALA synthetase can be suppressed when a drug which induces ALA synthetase also stimulates its own metabolism. Thus, induction of drug-metabolizing enzymes is suggested as an additional control mechanism influencing the induction of ALA synthetase.

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METHODS

Chemicals

m-Dichlorobenzene and 2,4-dichlorophenol (reagent grade) were purchased from Eastman Chemicals; bishydroxycoumarin (Dicumarol) from Abbott Laboratories (converted to the sodium salt by reflux with sodium hydroxide); hexobarbital from Winthrop Laboratories; and coproporphyrins I and III, and δ-aminolevulinic acid hydrochloride (ALA) from Calbiochemical Company.

Animals

Young Sherman strain female rats, 75–120 g, were fed regular laboratory chow and water *ad lib*., except where specifically stated. Animals were dosed by gastric intubation with *m*-dichlorobenzene (*m*-DCB) or 2,4-dichlorophenol (2,4-DCP) dissolved in peanut oil. The volume of drug administered was always 0·5 ml/100 g. Control animals were dosed with peanut oil.

For urinary porphyrin determinations, 24-hr urine collections were made with animals individually housed in metabolic cages. The urine was collected in light-protected, ice-chilled bottles.

Hexobarbital sleeping times were determined by administering 150 mg/kg of sodium hexobarbital i.p. and recording the time required for the return of the righting reflex.

Livers were fixed, sectioned and stained with hematoxylin and eosin for microscopic examination.

Bishydroxycoumarin metabolism

Twenty-four hr after the last dose of *m*-DCB or peanut oil, rats were injected with 20 mg/kg bishydroxycoumarin in saline i.p. and 5 hr later blood was drawn by cardiac puncture under ether anesthesia. The half-time of bishydroxycoumarin (20 mg/kg) in the male rat is reported to be 5 hr.[13] Bishydroxycoumarin levels were determined by the method of Nagashima *et al.*[14]

ALA synthesis in vitro

The ALA synthetase activity was measured in the whole liver homogenate by the method of Marver *et al.*[15] The reaction was terminated at 30 min by the addition of 0·5 ml 10% $ZnSO_4$ and 0·5 ml NaOH to each flask. Nine flasks were pooled for ALA analyses.

Analyses

Porphyrin determinations. Urinary coproporphyrin and uroporphyrin were determined by a modification of the method of Schlenker *et al.*[16] The eluting solution was changed to acetone–ether–glacial acetic–water (5:5:1:1), which gave a better recovery in our laboratory. The recoveries for coproporphyrin and uroporphyrin were 75–100 per cent and 60–80 per cent respectively. The porphyrin absorption was read on a Cary 14 recording spectrophotometer at 380 mμ, 430 mμ, and the maximum absorption peak. The extinction coefficients ($E^{\mu g/ml}_{1\,cm}$) used were 0·747 for coproporphyrin and 0·652 for uroporphyrin as listed by Schlenker *et al.*[16]

Coproporphyrin isomeric configuration was determined by ascending chromatography against de-esterified commerical coproporphyrins I and III in a lutidine–water system.[17]

ALA and porphobilinogen measurement. ALA and porphobilinogen (PBG) were determined by the method of Marver *et al.*[18] The ion-exchange columns and wash volumes were decreased to ⅜ size. After reacting the pyrroles with Erhlich's reagent, the ALA-pyrrole was read at 553 mμ with an extinction coefficient of 53, and the PBG chromagen at 555 mμ with an extinction coefficient of 68. Recovery of commercial ALA-HCl was usually 85–90 per cent.

Gas chromatography. The serum and liver concentrations of *m*-DCB and 2,4-DCP were determined by gas chromatography 6–7 hr after the last *m*-DCB administration. Blood was drawn by cardiac puncture under ether anesthesia. Slightly larger rats (200–230 g) were used because of the considerable quantity of serum required for analysis. Serum samples were acidified to pH 3, extracted with four parts of nanograde benzene, and the organic phase was dried with a small quantity of anhydrous sodium sulfate, parallel to the method of Dale *et al.*[19] Liver samples (0·7–1·8 g) were ground with a minimal amount of sodium sulfate by mortar and pestle, and then extracted with 20 ml benzene for 30 min on an automatic shaker. This sample preparation is similar to that described by Radomski and Fiserova-Bergerova.[20]

The gas chromatograph used was a Micro-Tek 2000 equipped with a tritium electron capture detector, dual channel E-2 electrometer, and 1 mV strip charter recorder.* The column used was a 6 ft × ⅛ in. U-shaped glass column packed with 15% FFAP† on 40/50 Chromosorb W. The operating parameters were as follows: inlet block, 210°; column oven, 130° (*m*-DCB), 190° (2,4-DCP); detector, 210°; carrier gas, prepurified nitrogen, 45 ml per min at 40 psi; and power source, d.c. mode of operation at 15 V. A polarizing voltage of 15 V gave a standing current of $2{\cdot}56 \times 10^{-9}$ amp at an electrometer setting of $10^2 \times 64$. Under these conditions, *m*-DCB and 2,4-DCP had retention times of 1·4 and 3·3 min respectively.

The response factor, ratio of nanograms injected to resulting peak height in millimetres, of each compound was determined by injecting 1–5 μl 2,4-DCP or *m*-DCB standards and measuring the resulting peak heights at an electrometer setting of $10^2 \times 8$. This attenuation on the electrometer was used throughout the analysis, except that aliquots of samples injected were varied to align with previous standard responses.

The lower limits of detectability of the method are based on a minimum peak height of 7 mm, which corresponds to a signal-to-noise ratio of 2. With this criterion, the minimum detectable amounts of *m*-DCB and 2,4-DCP were 0·6444 and 0·918 ng respectively.

The data were analyzed by Student's *t*-test.

RESULTS

Porphyrin excretion

Rats were dosed daily with 800 mg/kg of *m*-DCB. As shown in Fig. 1, excretion of urinary coproporphyrin was significantly higher than control levels on days 2, 3, 5 and 9. However, maximal stimulation occurred on day 3, and the excretion of coproporphyrin was significantly lower on days 5 and 9, despite continued daily administration of m-DCB.

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* Westronics Recorder, Westronics, Inc., Fort Worth, Tex.

† FFAP, Varian Aerograph, Walnut Creek, Calif.

Thin-layer chromatography of the urine revealed only the coproporphyrin III isomer. Uroporphyrin was not detected. Although the urinary excretion of ALA and PBG measured on days 1, 5 and 9 was significantly higher than that of controls, there was wide individual variation and no consistent trend between days 1 and 9.

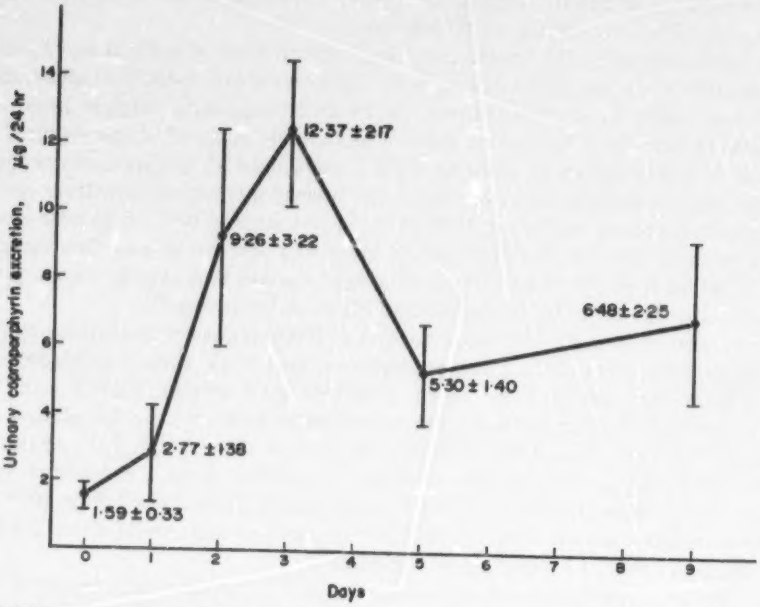

FIG. 1. Effect of *m*-DCB on urinary coproporphyrin excretion. Four female rats (90–120 g) were treated daily with 800 mg/kg *m*-DCB. The 24-hr urinary excretion of coproporphyrin (mean ± S. D.) is plotted versus time. The first dose was given on day 0, and the urinary coproporphyrin value for each day represents a urinary collection for the previous 24 hr. Urinary coproporphyrin excretion was significantly lower on days 5 and 9 than on day 3.

Livers from rats treated 1, 3 or 5 days with *m*-DCB (800 mg/kg) were essentially normal with minimal vacuolization when examined microscopically. There was no evidence of liver damage.

A few animals dosed with slightly larger amounts of *m*-DCB (900–1000 mg/kg) developed a more florid picture with gross coproporphyrinuria (>25 µg/24 hr), measurable uroporphyrinuria, and red staining of the periorbital hair and facial whiskers which fluoresced slightly under ultraviolet light.

The same biphasic pattern of urinary coproporphyrin excretion was observed in animals dosed daily with 2,4-DCP (800–1000 mg/kg), the major metabolite of *m*-DCB,[21] and with *p*-dichlorobenzene (900 mg/kg), a compound known to produce chemical porphyria in rats.[22]

ALA synthetase

Animals were dosed daily with *m*-DCB (800 mg/kg) or peanut oil for 1, 3 or 5 days and sacrified 24 hr after the last dose. The hepatic ALA synthetase activity *in vitro* is

TABLE 1. EFFECT OF *m*-DICHLOROBENZENE ON ALA SYNTHETASE ACTIVITY*

	ALA synthetase (mμmoles/g/hr)		
Treatment	Day 1	Day 3	Day 5
Control†	13·3 ± 4·4 (7)	18·8 ± 4·6 (6)	16·1 ± 4·8 (7)
m-DCB†	52·9 ± 15·3 (7)	40·5 ± 6·8 (7)	30·5 ± 6·2 (8)

* Female rats were treated orally for 1, 3 or 5 days with *m*-DCB (800 mg/kg) or peanut oil and sacrificed 24 hr after the last dose. All values represent the means ± S. D. The number of animals in each group is given in parentheses.

† ALA synthetase activity is lower on day 5 in *m*-DCB-treated animals than on day 1 ($P < 0{\cdot}01$) or day 3 ($P < 0{\cdot}02$).

reported in Table 1. The enzyme activity rose 3- to 4-fold after the first dose of *m*-DCB and then declined. All *m*-DCB-treated groups were significantly higher than controls. However, the ALA synthetase activity was significantly lower at 5 days than at 3 days ($P < 0{\cdot}01$) or at 1 day ($P < 0{\cdot}01$). ALA synthetase activity in control rats was 16·3 ± 6·4 mμmoles/g/hr ($N = 31$). None of the control groups in Table 1 varied significantly from this value.

One possibility for the decrease in ALA synthetase activity per gram of liver is the increase in liver size. However, Tables 1 and 2 show that the greatest increase in liver weight occured between days 1 and 3, while ALA synthetase activity per gram did not decrease significantly until day 5. Nor did the increase in liver size quantitatively account for the decrease in activity.

The degree of enzyme induction after a single dose of *m*-DCB is dose related (Table 3). In these experiments, rats were starved for 24 hr, dosed with *m*-DCB, and then starved until sacrificed. *m*-DCB produces maximal enzyme induction in starved rats at 800 mg/kg, and the induction of ALA synthetase activity is much greater than that produced by the same dose in fed rats (Table 1, day 1). The maximal stimulation caused by 2,4-DCP (445 mg/kg) under identical conditions was 3-fold (77 mμmoles/g/hr).

TABLE 2. EFFECT OF *m*-DICHLOROBENZENE ON LIVER WEIGHT*

Time	Treatment	Liver wt. (g)	Body wt. (g)	Liver/body wt. × 100
Day 1	Control	4·97 ± 0·63	121 ± 13	4·10 ± 0·22
	m-DCB	4·75 ± 0·67	111 ± 10	4·28 ± 0·24
		NS	NS	NS
Day 3	Control	5·57 ± 0·68	138 ± 9	4·04 ± 0·39
	m-DCB	6·49 ± 0·96	112 ± 11	5·77 ± 0·38
		(NS)	($P < 0{\cdot}001$)	($P < 0{\cdot}001$)
Day 5	Control	5·96 ± 0·62	143 ± 11	4·18 ± 0·31
	m-DCB	7·31 ± 0·87	117 ± 11	6·24 ± 0·49
		($P < 0{\cdot}01$)	($P < 0{\cdot}01$)	($P < 0{\cdot}001$)

* Data are from animals in Table 1. NS = not significant.

Table 3. Effect of varying doses of *m*-dichlorobenzene on ALA synthetase in the fasted rat*

Treatment (mg/kg)	No. of animals	ALA synthetase activity (mμmoles/g/hr)
Control	4	21·0 ± 3·9
m-DCB		
200	3	49·1 ± 13·1
400	4	73·8 ± 12·3
800	4	125·1 ± 27·0
1600	4	39·1 ± 18·7

* Female rats (90–120 g) were starved for 24 hr and then dosed orally with *m*-DCB or peanut oil. Starvation continued until sacrifice 24 hr later. All values represent the means ± S. D.

These experiments demonstrate that daily oral dosage with *m*-DCB results in induction of hepatic ALA synthetase and an increase in urinary coproporphyrin excretion, which peak at 1 and 3 days, respectively, while both parameters are significantly lower by 5 days. The adaptation, or tolerance, to *m*-DCB suggested that the animals might be detoxifying the drug more rapidly. This in turn would lower the effective concentration of *m*-DCB, or its metabolites, which stimulates ALA synthetase.

Metabolism of hexobarbital and bishydroxycoumarin

To test the possibility that *m*-DCB stimulates its own detoxification, presumably by induction of the drug-metabolizing enzymes in the smooth endoplasmic reticulum, we first studied the effect of *m*-DCB on the metabolism of hexobarbital and sodium bishydroxycoumarin.

Young female rats were treated for 1, 3 or 5 days with *m*-DCB or peanut oil. As seen in Fig. 2, the hexobarbital sleeping times were significantly shortened after one dose of *m*-DCB ($P < 0{\cdot}02$), and by 3 days were only 20 per cent of control ($P < 0{\cdot}001$).

To study the effect of *m*-DCB on the rate of metabolism of bishydroxycoumarin, rats were treated as described above. Five hr after bishydroxycoumarin administration, the serum concentrations of bishydroxycoumarin were significantly lower in rats treated with *m*-DCB for 5 days than in controls (Table 4, $P < 0{\cdot}02$).

Serum and liver concentrations of m-*DCB and* 2,4-*DCP*

The above experiments suggest that *m*-DCB does stimulate the hepatic degradation of some drugs. The following experiments were designed to test the hypothesis that *m*-DCB stimulates its own detoxification and thus lowers the effective drug concentration. Since we have shown that 2,4-DCP, the major metabolite of *m*-DCB in the rabbit, also stimulates ALA synthetase and coproporphyrin excretion, it was necessary to measure both 2,4-DCP and *m*-DCB simultaneously after *m*-DCB administration.

Liver and serum samples were analyzed as described in Methods. The efficacy of the extraction procedures was tested by fortifying serum and liver samples from control rats with *m*-DCB and 2,4-DCP in the concentration range detected in the dosed animals. The *m*-DCB recoveries are presented in Table 5. The 2,4-DCP recoveries were 65–89 per cent in serum and 93–100 per cent in the liver.

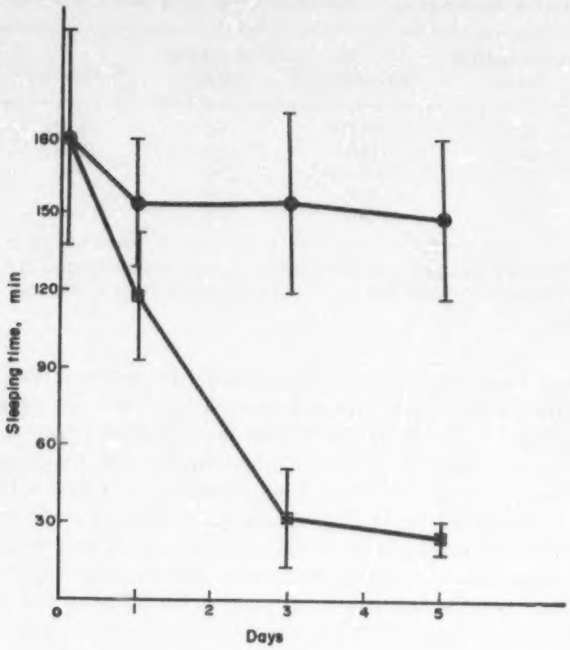

FIG. 2. Effect of *m*-DCB on hexobarbital sleeping time. Female rats (75–100 g) were treated for 0, 1, 3 or 5 days with *m*-DCB (800 mg/kg) or peanut oil. They were injected with 150 mg/kg of sodium hexobarbital i.p. 24 hr later, and sleeping times were measured. All points represent the means ± S. D. of seven or eight animals. Sleeping times are significantly reduced by *m*-DCB at days 1 ($P < 0{\cdot}02$), 3 ($P < 0{\cdot}001$) and 5 ($P < 0{\cdot}001$). Control animals, ○—○; *m*-DCB-treated animals, □—□.

TABLE 4. EFFECT OF *m*-DICHLOROBENZENE ON BISHYDROXYCOUMARIN METABOLISM*

Treatment	Serum bishydroxycoumarin concn (μg/ml)		
	Day 1	Day 3	Day 5
Control	107 ± 16 (4)	120 ± 45 (4)	133 ± 10 (4)
m-DCB	94 ± 27 (4)	78 ± 36 (4)	82 ± 29† (3)

* Female rats were treated with *m*-DCB (800 mg/kg) or peanut oil for the times indicated. Twenty-four hr after the last dose, sodium bishydroxycoumarin was administered i.p., and 5 hr later the animals were anesthetized with ether and bled by cardiac puncture. Each value represents the mean ±S. D. The number of animals in each group is given in parentheses.

† $P < 0{\cdot}025$.

TABLE 5. RECOVERY OF *m*-DICHLOROBENZENE FROM LIVER AND SERUM*

Serum *m*-DCB (μg/ml)	% Recovery	Liver *m*-DCB (μg/g)	% Recovery
1·288	94·9	6·0	110·2
3·386	110·7	12·0	106·4
5·152	104·4	30·9	94·2
7·728	86·0	41·8	110·0
9·016	83·4	63·0	81·6

* *m*-DCB was added to liver or serum samples from untreated rats in the amounts listed above. All values are the average of duplicate runs.

Rats were treated daily with 1, 3 or 5 doses of m-DCB. As seen in Table 6, the serum *m*-DCB concentration was higher on day 3 than on day 1, but was significantly lower on day 5 than on day 3 ($P < 0·02$). The hepatic concentration of *m*-DCB showed the same pattern, but the difference between the concentration at 3 days and 5 days was not quite significant ($0·10 > P > 0·05$). Rats pretreated for 4 days with 40 mg/kg of phenobarbital, a known inducer of drug metabolism, before a single dose of m-DCB had slightly lower concentrations of *m*-DCB in the serum and liver than animals given only the single dose of *m*-DCB, but neither difference was significant. 2,4-DCP could not be detected in the serum or liver of any of the animals. The detectable level depended on the size of the sample taken (serum, <0·026–0·083 μg/ml; liver, <0·74–3·4 μg/g).

TABLE 6. EFFECT OF DAILY DOSAGE ON THE LEVEL OF *m*-DICHLOROBENZENE IN LIVER AND SERUM*

Treatment	Serum *m*-DCB (μg/ml)	Liver *m*-DCB (μg/g wet wt.)	Liver wt. (g)	Body wt. (g)
1 dose *m*-DCB	3·25 ± 1·31	13·01 ± 6·92	7·62 ± 0·51	230 ± 10
3 doses *m*-DCB	8·89 ± 1·66	44·17 ± 6·31	9·67 ± 0·22	202 ± 13
5 doses *m*-DCB	5·91 ± 2·02	32·10 ± 16·18	12·14 ± 1·46	193 ± 11
4 doses phenobarbital × 1 dose *m*-DCB	2·24 ± 0·49	8·49 ± 2·37	9·17 ± 0·41	235 ± 7

* Female rats (200–230 g) were treated orally with *m*-DCB (800 mg/kg) for 1, 3 or 5 days; or phenobarbital (40 mg/kg) i.p. for 4 days followed by 1 dose of *m*-DCB on the fifth day. The animals were anesthetized with ether 6–7 hr after the last dose of *m*-DCB, and blood was drawn by cardiac puncture. All values represent the means ±S. D. of six animals.

DISCUSSION

The administration of *m*-DCB daily to rats induces ALA synthetase. At large doses (900–1000 mg/kg), this drug produces experimental hepatic porphyria similar to that described by Rimington and Ziegler[22] with *p*-dichlorobenzene. However, with daily doses of 800 mg/kg of *m*-DCB, we observed a biphasic stimulation in the excretion of urinary coproporphyrin and ALA synthetase activity, which peaked by 3 days and then declined. Drug metabolism was also stimulated by *m*-DCB. The increase in ALA synthetase and the changes in drug metabolism reported above were not parallel. In fact, the decrease in urinary excretion of porphyrins and in ALA synthetase activity at 5 days coincided with the time of maximal stimulation of drug metabolism occurring with this dosage. These data suggest that the decrease in ALA synthetase after 3 days may be a result of increased metabolism of *m*-DCB by the liver microsomal systems.

The serum concentration of *m*-DCB peaked on day 3 and was significantly lower by day 5, supporting the hypothesis that the decrease in ALA synthetase is a result of increased metabolism of *m*-DCB. It should be noted that the serum concentrations of *m*-DCB did not strictly parallel ALA synthetase activity. Although the serum concentration of *m*-DCB was decreasing by day 5, it was still higher on day 5 than on day 1. In contrast, induction of ALA synthetase was maximal by day 1. However, ALA synthetase was measured 24 hr after the last dose of *m*-DCB, while the serum levels of *m*-DCB were measured 6–7 hr after the last dose.

The porphyria seen after *m*-DCB could be caused by either *m*-DCB or a metabolite. Parke and Williams[21] demonstrated that *m*-DCB is oxidized in rabbits mainly to 2,4-DCP, which is excreted as conjugates of glucuronic and sulfuric acids. 2,4-DCP has been implicated as a causative agent of porphyria by Bleiberg *et al.*,[23] and we have found that it causes a biphasic excretion of coproporphyrin similar to that seen after *m*-DCB administration.

We found no 2,4-DCP present in the liver or serum of animals treated with *m*-DCB. If 2,4-DCP is a metabolite of *m*-DCB in the rat, the circulating level is less than 3 per cent of the *m*-DCB present. Therefore, it probably does not contribute to the observed increase in ALA synthetase seen after *m*-DCB administration.

Alternative explanations for the decrease in ALA synthetase are possible: (1) The relatively high serum levels of *m*-DCB which occur after three doses may cause liver damage and nonspecific depression of enzyme activities. This possibility is not supported by morphological examination of the tissue or by the fact that activity of the liver drug-metabolizing enzymes is higher at this time. In addition, a higher dose of *m*-DCB caused greater porphyrinuria when administered daily. (2) Administration of the drug could result in increased production of repressors or inhibitors of ALA synthetase. Heme, hemoglobin, hemin and bilirubin have been shown to inhibit induction of ALA synthetase.[5,24] Damage to the red blood cells or the liver could increase the concentration of one of these known inhibitors. We found no increase in the serum bilirubin levels or any suggestion of hemolysis after 5 days of *m*-DCB treatment. However, the production of suppressive compounds during porphyria is not ruled out.

Induction of drug-metabolizing enzymes has been reported to be accompanied by an increase in the level of the terminal oxidase, cytochrome P-450, and the changes are often parallel.[10] Drugs which induce P-450 and liver microsomal drug metabolism apparently stimulate heme synthesis in the liver, since blocking heme synthesis by administration of heme or 3-amino-1,2,4-triazole prevents phenobarbital induction of P-450.[6,12] In addition, drug-mediated increases in cytochrome P-450 are blocked by a variety of inhibitors of RNA and protein synthesis, suggesting that the increase in cytochrome P-450 is a result of *de novo* protein synthesis.[10,25] Thus, the several-fold increase in P-450 seen with induction of drug-metabolizing enzymes may be accompanied by induction of the rate-limiting enzyme, ALA synthetase.[9, 10] The induction of ALA synthetase by known inducers of drug metabolism is not unexpected, since P-450 accounts for more than 50 per cent of the heme synthesized in the normal liver.[8] However, the control of induction of drug metabolism and ALA synthetase is not completely understood, since many of the strongest inducers of drug metabolism are merely weak inducers of ALA synthetase and vice versa.[9]

The results reported in this paper suggest that *m*-DCB stimulated its own degradation, and repeated dosage resulted in lower plasma *m*-DCB levels than a single dose. This diminished the induction of ALA synthetase and lessened the porphyrogenic response. We suggest that many drugs may be potentially porphyrogenic, but porphyrogenic activity is limited by the ability of the drug to increase its own metabolism.

Addendum—Since this manuscript was submitted for publication, several reports have been published which confirm our observations. Moore *et al.*[26] have demonstrated that daily administration of phenobarbital to rats results in a maximal rise in hepatic δ-ALA synthetase activity at about 4 days and then a fall off in activity at a time when stimulation of hepatic drug metabolism reaches a maximum.[27] Kaufman *et al.*[28] have found that pretreatment of rats with phenobarbital abolished the ability of progesterone or AIA to induce δ-ALA synthetase and increased the rate of metabolism of AIA. The ability of a drug to induce chemical porphyria appears to depend on the balance of its ability to induce δ-ALA synthetase and the rate of inactivation of the inducing drug. It is interesting to note that hexachlorobenzene, which is not metabolized,[29] is one of the few drugs which produces a sustained induction of δ-ALA synthetase.[30]

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Atlanta, Ga. 30333

Chlorinated Hydrocarbon Insecticides in Plasma and Milk of Pregnant and Lactating Women

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Five pregnant women were studied for chlorinated hydrocarbon insecticide residues in their blood and milk. Blood samples were obtained from each between 30 days of pregnancy and 115 days postpartum. Three milk samples were collected from each between three and 96 days postpartum. Sample extracts of plasma and milk were analyzed by electron-capture gas-liquid chromatography for chlorinated hydrocarbon insecticides. Eight chlorinated insecticides were detected. The concentrations of these compounds in plasma were within the lower part of the range previously reported for nonpregnant women. A small but statistically significant decrease in the plasma concentration of *p,p'*-DDT, *p,p'*-DDE, *o,p'*-DDE, *p,p'*-DDD, and total BHC was observed one to six days postpartum. The concentration of the detected compounds in milk varied greatly among the individual women but was within the range reported by others.

IN 1951, Laug et al[1] found DDT (1,1,1-trichloro-2,2-bis [*p*-chlorophenyl]-ethane) in 30 of 32 human milk samples collected in the Washington, DC area. Other reports from California,[2] Hungary,[3] and Russia[4] followed. Quinby et al[5] reported DDT in individual and pooled samples of milk from women in Chicago, Wenatchee, Wash, and Phoenix, Ariz. The presence of DDT, its metabolites, and other chlorinated hydrocarbon insecticides—aldrin (1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4, *endo*, *exo*-5,8-dimethanonaphthalene), dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4 *endo*, *exo*-5,8-dimethanonaphthalene), and benzene hexachloride (BHC) (1,2,3,4,5,6-hexachlorocyclohexane)—in human milk was also reported from England,[6] Hungary,[7] and Italy.[8] Except for Damaskin[4] who found 1.22 ppm to 4.88 ppm DDT in 16 milk samples, all investigations reported comparable levels of DDT in milk ranging from about 0.05 to 0.26 ppm.

Dale et al[9] from this laboratory reported the levels of several chlorinated hydrocarbons in plasma of men and women of the general population. No data on the blood levels of chlorinated hydrocarbon insecticides in women during the gestation and lactation period were available, nor was it known whether concentrations of these compounds in human milk vary during the early and late lactation period. Two studies were undertaken to gain information on the levels of insecticides in women during pregnancy and lactation. One investigation established the levels of chlorinated hydrocarbon insecticides in the plasma of women during early and late pregnancy, and in the plasma and milk for three months postpartum. In this

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attitudes concerning the marital relationship. Even in the modern 1960's, a majority of men cannot accept a wife who does not place the home and family first. Too often, mutual agreement between the young married couple regarding the wife's freedom to continue her studies or work is rapidly forgotten as the young husband faces dull dinners, unwashed shirts and a very tired wife.

Women who enter marriage, secure in the naive conviction that nothing need interfere with the pursuance of their careers, endanger their marriages and their happiness. If they do not come to a realization of their obligations to their families, they often establish a life style in which husband and children are subjugated to the narcissistic functioning of "mama." Most career women recognize the need to adjust their work efforts in terms of their families. I know highly trained professional women who have refused desirable positions requiring them to be at work on the husband's day at home. Countless career women arrange their schedules in order to be available as chauffeur for a child's art, music, or dancing classes. Such maneuvering is considered necessary, particularly during the early marriage and child-rearing years. The deferment of personal career ambitions to the later years when growing independence of children allows for increased flexibility in the woman's life is the modus operandi among the women in active careers today. Women know that there *is* no such thing as successful integration of an all-out career and mothering.

Judging from the reluctance of bright, highly educated females to continue their professional work following the birth of a baby, the desire to "mother" is still a tremendously strong drive in young women. Indeed, it appears that there has been little change in the forces that shape and support the female image of what constitutes sex-appropriate roles. The college woman today is very much her Mother's daughter! We see the talented and educated college student choosing to teach history, for example, rather than become a lawyer because "law is a man's profession and, in any case, teaching permits time to be a wife and mother." My own generation of college women turned out thousands of female public school teachers for similar reasons. The choice of a career was determined largely and, perhaps, wisely by how well it would harmonize with the future roles of wife and mother. Then, as now, the young women who dared to enter the fields of medicine, law, and the sciences were not necessarily the most talented nor the brightest of the college students. Rather, they were, and continue to be, most highly motivated and determined. Certainly the small number of women entering our medical schools today cannot be explained entirely on misogynic prejudice nor lack of opportunity. There is an undeniable and understandable reluctance among many young women to enter demanding careers which could place them into frustrating and conflicting positions. Always, and despite the outcry of the purveyors of "feminine mystique," most women develop their interests and set their work goals as their life situations demand. In sharp contrast, the man structures his life situation in ways which further support his specified career.

This difference in perspective was recently illustrated by an eminent woman physician, who, upon being late for an appointment, simply explained, "My husband's car wouldn't start, so he took mine."

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Table 1.—*Obstetrical History and Drug Therapy of the Pregnant Women*

Patient	Age	Occupation	No. of Pregnancies	Delivery	Medication in Hospital: Prior to Delivery	Medication in Hospital: After Delivery
Study 1						
1	30	Housewife	4	Cesarean	Spinal anesthesia	Meperidine Propoxyphene hydrochloride Secobarbital
2	21	Laboratory technician	1	Normal	Meperidine Scopolamine Promethazine Pentobarbital	Propoxyphene hydrochloride Secobarbital Ergonovine maleate
3	33	Physician	3	Normal	Propiomazine hydrochloride Chlorpromazine	Ergonovine maleate
4	20	Laboratory technician	1	Normal	Meperidine Scopolamine Levallorphan	Ergonovine maleate Secobarbital
5	22	Laboratory technician	2	Normal	Meperidine Scopolamine Propiomazine hydrochloride Levallorphan	Ergonovine maleate Secobarbital
Study 2						
6	25	Housewife	2	Normal	None	Propoxyphene hydrochloride Ergonovine maleate
7	26	Statistical assistant	2	Normal	Meperidine Promethazine Scopolamine	Propoxyphene hydrochloride Ergonovine maleate

study, a change in the concentration of these compounds in plasma was observed within the period of 30 days before to 55 days after delivery. In a second study, plasma from two additional subjects was examined during the period six days before to 30 days after delivery in order to determine the concentrations of these compounds prior to and immediately after delivery at closer intervals. This paper reports the results of these two studies.

Materials and Methods

Both groups (study 1 and 2) consisted of healthy women of the same socioeconomic group and metropolitan area, who had no known special exposure to pesticides. Table 1 presents their age, obstetrical history, and profession.

Study 1.—Five women participated in this study. Individual blood samples were drawn from 240 to 120 and 30 to 7 days before delivery and between one to six, 36 to 55, and 90 to 115 days postpartum. The first milk samples were collected three to six days postpartum. Subsequent milk samples were collected simultaneously with the other two postpartum blood samples. All blood samples were obtained before lunch except the samples of patient 1 which were obtained in the evening. The first blood sample and the last milk sample were not collected from one participant (patient 2) (Table 2 and 3).

Study 2.—Two women were subjects in this study in which only blood samples were obtained. These samples were drawn at six and seven days before delivery, at delivery, and at 6, 13 to 15, and 27 to 30 days postpartum. Cord blood was used as the sample at delivery. Milk samples were not collected in this study.

The blood samples from all subjects studied were collected in 10-ml tubes which contained potassium oxalate as an anticoagulant. The plasma was separated and refrigerated prior to analysis. These samples were analyzed by the method of Dale et al[10] with the exception of a change in the column loading, column size, and nitrogen flow rates. The column consisted of an aluminum tube, 6 feet × ¼ inch, packed with 3% diethylene glycol succinate. The carrier gas flow was 40 cc/min, and no scavenger gas was used.

Controls.—Blood was collected from ten nonpregnant women of comparable socioeconomic status and of the same city. The plasma of this group was analyzed for chlorinated hydrocarbon insecticides by the same method and the results compared with those obtained for the pregnant women in study 1.

The milk samples were collected by manually pumping the breast and were frozen for storage prior to analysis. Whole milk (0.2 ml) was pipetted into a ground-glass stoppered centrifuge tube. The sample was then partitioned with 2 ml of methanol and 3 ml of 1% potassium carbonate. The sample was extracted three times with 3 ml of *n*-hexane. The combined *n*-hexane extracts were concentrated to a volume of 50μl or 100μl. An appropriate aliquot from the 50μl or 100μl *n*-hexane concentrate was analyzed by electron-capture gas-liquid chromatography.

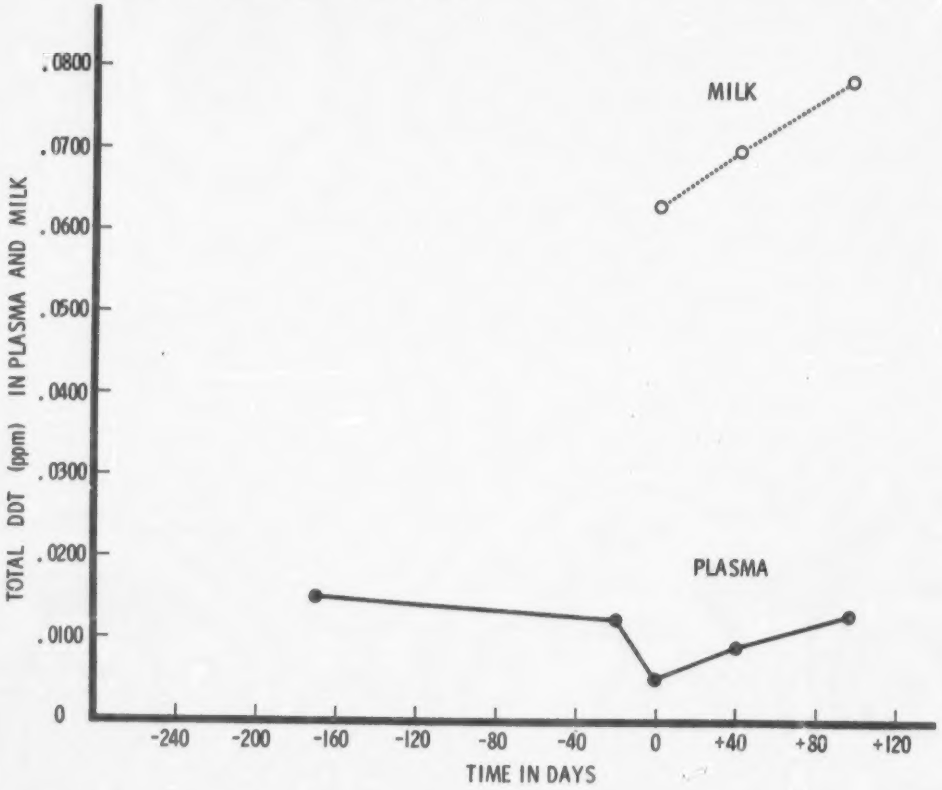
Results

Study 1.—Table 2 presents the analytical results for *p,p′*-DDT, *o,p′*-DDT, *p,p′*-DDE, *o,p′*-DDE, *p,p′*-DDD, total DDT (all isomers, metabolites, and analogues), total BHC, heptachlor epoxide, and dieldrin in plasma at various intervals during pregnancy and after delivery. Table 3 gives concentrations of these same chlorinated insecticides found in the milk samples at various intervals after delivery. Figure 1 illustrates the mean concentrations in parts per million of total DDT in the plasma and milk at various intervals during pregnancy and after delivery.

The concentrations of the detected chlorinated hydrocarbon insecticides in the plasma of the pregnant women were within the range previously reported[9] for healthy nonpregnant women in childbearing age of the same socioeconomic group and metropolitan area. The values for ten nonpregnant women were included in Table 2 for comparison. These volunteers also had no known special exposure to pesticides.

The plasma concentration of *p,p′*-DDT and its metabolites showed a small decrease

Fig 1.—Mean concentration of total DDT (ppm) in plasma of five women at different intervals during pregnancy and in plasma and milk after delivery.



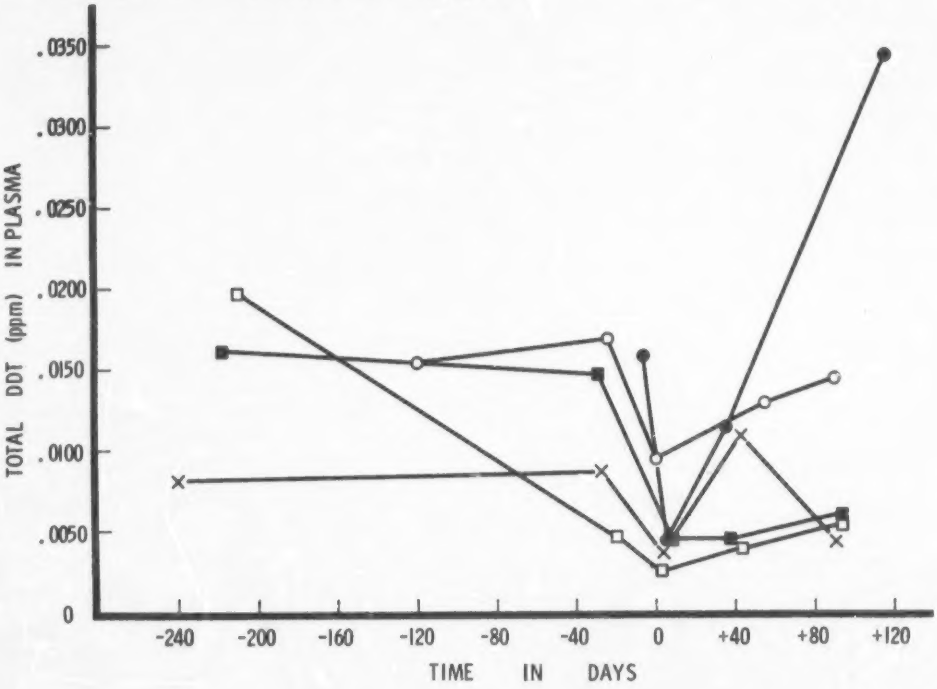
at or soon after delivery. A decrease in the concentration of total BHC in plasma was also observed during this period. The observed decrease of the plasma level of the various chlorinated hydrocarbon insecticides was only significant for the *p,p'*-DDE fraction ($P < 0.05$) and for the sum of the DDT fractions ($P < 0.01$). Subsequently, the concentration of these compounds increased to approximately the levels found during pregnancy. The concentration of *o,p'*-DDT did not show this trend.

Figure 2 to 4 illustrate the small decrease of total DDT, *p,p'*-DDT, and *p,p'*-DDE in the plasma of five individuals at one to six days postpartum. The curves indicate corresponding plasma levels for these compounds in the five subjects. The individual curves (Fig 5) for the plasma levels of dieldrin did not correspond as well to each other.

The mean total DDT concentrations in milk increased during the lactation period (Fig 1). The individual levels of the total DDT, *p,p'*-DDT, and *p,p'*-DDE (Fig 6 to 8) concentration show pronounced variation at the different intervals tested. The range of the total DDT levels falls within the limits reported by others.[5] The mean concentration for *p,p'*-DDT decreases somewhat during lactation while that of *p,p'*-DDE increases. However, this was not statistically significant since the individual variation was very pronounced. Traces of α-, γ-, and β-BHC, heptachlor epoxide, and dieldrin were also observed in the milk samples. Individual variation was found for these chlorinated hydrocarbon insecticides, too. The values obtained for dieldrin and total BHC are comparable to those reported in England.[6]

Study 2.—Table 4 presents the concentrations of DDT, DDT and related materials, total BHC, heptachlor epoxide, and dieldrin in the plasma of the two women. The concentrations of all the chlorinated compounds detected were similar to those found in study 1. A statistical comparison of the mean values for the total DDT concentration in plasma obtained before delivery with

Fig 2.—Individual concentration of total DDT (ppm) in plasma of five women at different intervals during pregnancy and after delivery. **Open circle** indicates patient 1; **solid circle**, patient 2; **open square**, patient 3; **solid square**, patient 4; and **x**, patient 5.



the first plasma sample of study 1 is not significantly different. However, the sample taken at delivery ($P < 0.025$) and at 14 ($P < 0.05$) and 30 ($P < 0.05$) days after delivery showed a slight but significantly lower total DDT concentration than the first sample of study 1. At six days after delivery, a significant difference in the total DDT concentration of plasma could not be demonstrated when comparing the results of study 2 with the results of the first samples of study 1. A significant difference between the concentration of the other measured compounds was also not observed when the results of study 2 were compared with the results of the first sample of study 1.

Table 2.—Concentration* of Chlorinated Insecticides and Related Compounds in Plasma of

Days†		*p,p'*-DDT	*o,p'*-DDT	*p,p'*-DDE	*o,p'*-DDE
−240 to	Range	0.0009-0.0046	<0.0001-0.0019	0.0027-0.0132	<0.0001-0.0014
−120 Days	Mean	0.0032	0.0007	0.0084	0.0004
N=4	SE±	0.0009	0.0004	0.0022	0.0003
−30 to	Range	0.0013-0.0068	<0.0001-0.0074	0.0027-0.0092	<0.0001-0.0002
−7 Days	Mean	0.0031	0.0029	0.0052	0.0001
N=5	SE±	0.0011	0.0013	0.0012	0.00005
+1 to	Range	0.0007-0.0032	<0.0001-0.0006	0.0012-0.0051	<0.0001-0.0002
+6 Days	Mean	0.0019	0.0002	0.0024	0.0001
N=5	SE±	0.0005	0.0001	0.0007	0.0004
+35 to	Range	0.0010-0.0044	<0.0001-0.0008	0.0022-0.0068	<0.0001-0.0007
+55 Days	Mean	0.0025	0.0002	0.0048	0.0003
N=5	SE±	0.0006	0.0001	0.0010	0.0001
+90 to	Range	0.0006-0.0063	<0.0001-0.0003	0.0014-0.0090	<0.0001-0.0020
+115 Days	Mean	0.0033	0.0001	0.0045	0.0005
N=5	SE±	0.0010	0.00006	0.0013	0.0004
Females of	Range	0.0026-0.0095	<0.0001-0.0011	0.0050-0.0203	<0.0001
general population	Mean	0.0053	0.0007	0.0130	—
N=10	SE±	0.0007	0.0001	0.0015	—

* Parts per million.

† The delivery day was designated as day 0. Counting backwards from day 0, the days of pregnancy were designated as (—) days. The days postpartum were designated as (+) days counting from the day of delivery.

Table 3.—Concentration* of Chlorinated Insecticides and Related Compounds in Milk

Days†		*p,p'*-DDT	*o,p'*-DDT	*p,p'*-DDE	*o,p'*-DDE
+3 to	Range	0.0146-0.0890	<0.0001-0.0072	0.0052-0.0268	<0.0001-0.0027
+6 Days	Mean	0.0341	0.0027	0.0177	0.0007
N=5	SE±	0.0138	0.0012	0.0038	0.0005
+37 to	Range	0.0078-0.0586	<0.0001-0.0036	0.0187-0.0435	<0.0001-0.0022
+60 Days	Mean	0.0328	0.0012	0.0274	0.0010
N=5	SE±	0.0107	0.0007	0.0049	0.0004
+90 to	Range	0.0137-0.0306	<0.0001-0.0108	0.0193-0.0981	<0.0001-0.0028
+96 Days	Mean	0.0223	0.0037	0.0411	0.0010
N=4	SE±	0.0040	0.0025	0.0190	0.0007

* Parts per million.

† The delivery day was designated as day 0. Counting backwards from day 0, the days of pregnancy were designated as (—) days. The days postpartum were designated as (+) days counting from the day of delivery.

Table 4.—Concentrations* of Chlorinated Insecticides and Related Compounds

Days Before or After Delivery†	*p,p'*-DDT	*o,p'*-DDT	*p,p'*-DDE	*o,p'*-DDE
−6 to −7 days	0.0010-0.0068	<0.0001	0.0012-0.0021	0.0008-0.0015
Delivery day	<0.0001-0.0006	<0.0001	0.0010-0.0047	<0.0001-0.0001
+6 days	0.0012-0.0222	<0.0001-0.0126	0.0013-0.0043	<0.0001
+13 to +15 days	0.0005-0.0015	<0.0001	0.0004-0.0036	<0.0001
+27 to +30 days	0.0008-0.0016	<0.0001	0.0016-0.0026	<0.0001

* Parts per million.

† The delivery day was designated as day 0. Counting backwards from day 0, the days of pregnancy were designated as (—) days. The days postpartum were designated as (+) days counting from the day of delivery.

‡ The values were the same for both women.

Comment

The results in the present study show that the concentration of chlorinated hydrocarbon insecticides in plasma of pregnant and lactating women falls within the same range as that of nonpregnant women.

The mean plasma concentrations of *p,p'*-DDT and all DDT derived compounds, and total BHC showed a small but significant decrease at or soon after delivery and subsequently increased to the levels observed during early pregnancy. Dieldrin followed a similar trend. Despite the individual variation, the mean concentration of *p,p'*-DDT, its isomers and metabolites reported as total

Five Women in Study 1 at Various Intervals Before and After Delivery

p,p'-DDD	Total as DDT	Total BHC	Heptachlor Epoxide	Dieldrin
<0.0001-0.0027	0.0081-0.0197	0.0015-0.0097	<0.0001-0.0019	0.0004-0.0020
0.0011	0.0149	0.0045	0.0005	0.0010
0.0006	0.0024	0.0019	0.0004	0.0003
<0.0001-0.0010	0.0047-0.0170	0.0008-0.0034	0.0002-0.0015	0.0008-0.0030
0.0003	0.0122	0.0023	0.0007	0.0014
0.0002	0.0024	0.0005	0.0003	0.0004
<0.0001-0.0005	0.0025-0.0095	<0.0001-0.0044	<0.0001-0.0033	<0.0001-0.0024
0.0001	0.0050	0.0012	0.0008	0.0009
0.0001	0.0012	0.0008	0.0006	0.0004
<0.0001-0.0011	0.0040-0.0130	<0.0001-0.0023	0.0001-0.0006	0.0003-0.0061
0.0004	0.0088	0.0011	0.0003	0.0018
0.0002	0.0019	0.0004	0.0001	0.0010
<0.0001-0.0003	0.0044-0.0152	0.0006-0.0028	<0.0001-0.0005	0.0003-0.0010
0.0001	0.0091	0.0016	0.0002	0.0007
0.00006	0.0023	0.0004	0.00009	0.0001
<0.0001	0.0088-0.0295	0.0014-0.0096	0.0003-0.0010	0.0001-0.0012
—	0.0205	0.0034	0.0006	0.0003
—	0.0022	0.0008	0.0001	0.0001

of Five Women in Study 1 at Various Intervals After Delivery

p,p'-DDD	Total as DDT	Total BHC	Heptachlor Epoxide	Dieldrin
<0.0001-0.0140	0.0500-0.0990	0.0006-0.0157	<0.0001-0.0024	0.0030-0.0137
0.0053	0.0632	0.0075	0.0010	0.0069
0.0024	0.0090	0.0029	0.0004	0.0020
<0.0001-0.0104	0.0422-0.1127	<0.0001-0.0202	<0.0001-0.0032	0.0034-0.0074
0.0039	0.0699	0.0098	0.0014	0.0045
0.0018	0.0127	0.0032	0.0005	0.0007
<0.0001-0.0080	0.0404-0.1563	<0.0001-0.0047	<0.0001-0.0044	0.0029-0.0146
0.0049	0.0784	0.0024	0.0027	0.0073
0.0018	0.0263	0.0010	0.0009	0.0025

in the Plasma of Two Women in Study 2 Before and After Delivery

p,p'-DDD	Total as DDT	Total HBC	Heptachlor Epoxide	Dieldrin
<0.0001	0.0042-0.0098	0.0008-0.0010	<0.0001	<0.0001-0.0002
<0.0001	0.0017‡	<0.0001-0.0015	<0.0001	<0.0001-0.0003
<0.0001	0.0026-0.0395	0.0019-0.0029	<0.0001-0.0002	<0.0001
<0.0001	0.0009-0.0055	0.0011-0.0025	<0.0001	<0.0001-0.0008
<0.0001	0.0034-0.0037	0.0007-0.0016	<0.0001	<0.0001

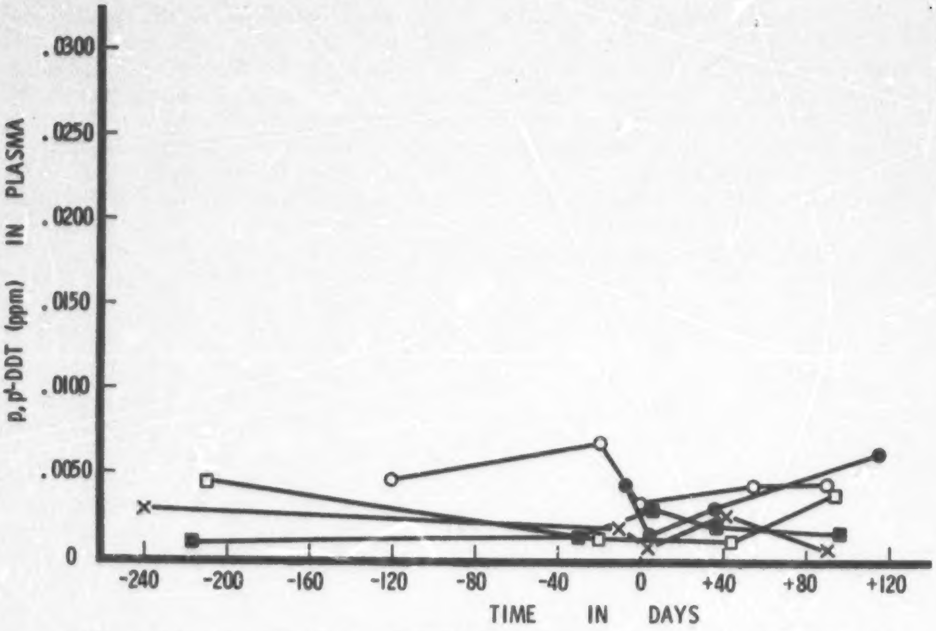
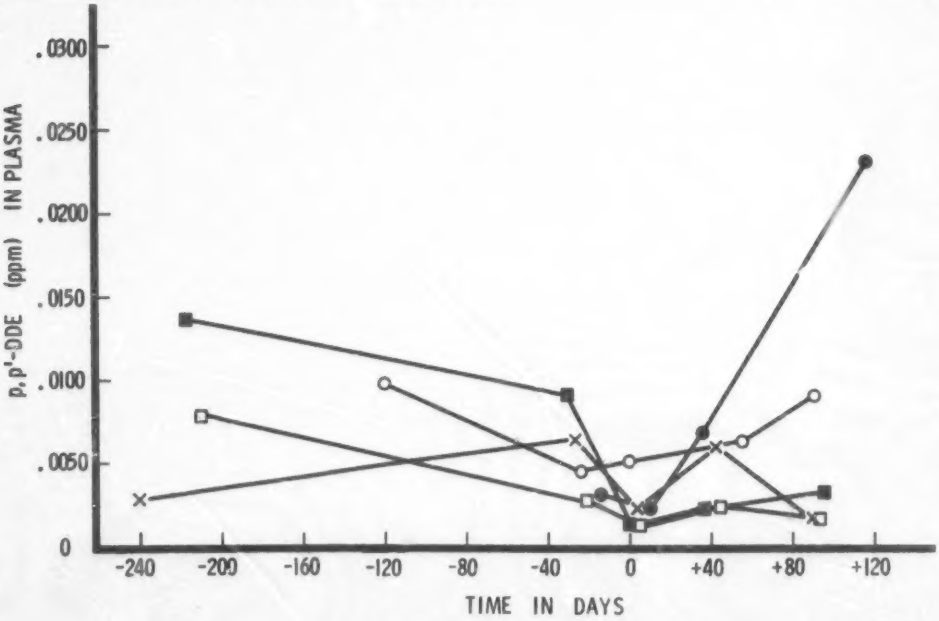


Fig 3.—Individual concentration of p,p'-DDT (ppm) in plasma of five women at different intervals during pregnancy and after delivery. Open circle indicates patient 1; solid circle, patient 2; open square; patient 3; solid square, patient 4; and x, patient 5.

Fig 4.—Individual concentration of p,p'-DDE (ppm) in plasma of five women at different intervals during pregnancy and after delivery. Open circle indicates patient 1; solid circle, patient 2; open square, patient 3; solid square, patient 4; and x, patient 5.



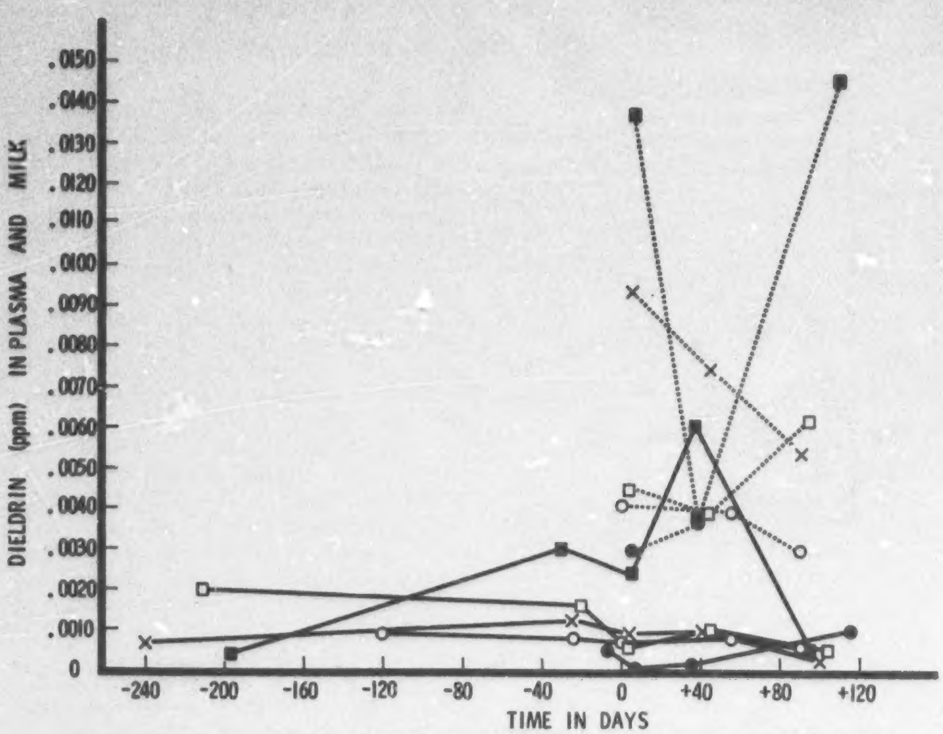


Fig 5.—Individual concentration of dieldrin (ppm) in plasma of five women at different intervals during pregnancy and in plasma (solid line) and milk (broken line) after delivery. Open circle indicates patient 1; solid circle, patient 2; open square, patient 3; solid square, patient 4; and x, patient 5.

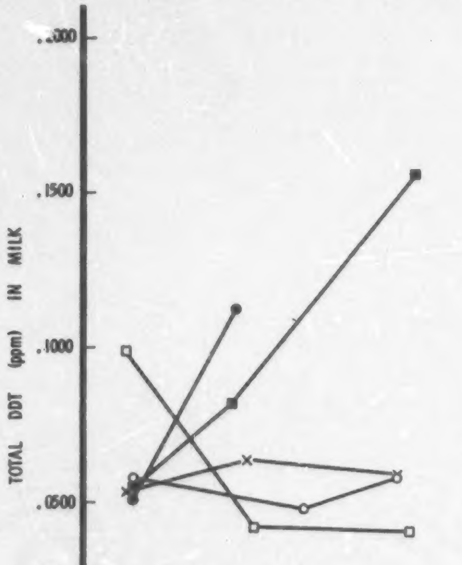
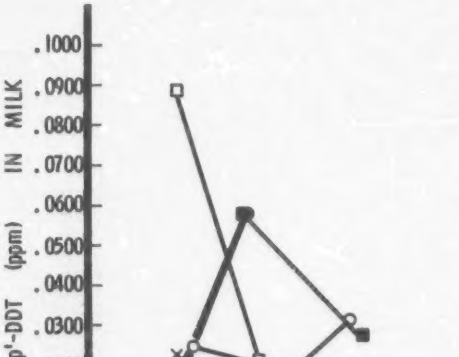


Fig 6.—Individual total DDT in milk after delivery. Open circle indicates patient 1; solid circle, patient 2; open square, patient 3; solid square, patient 4; and x, patient 5.

Fig 7.—Individual concentration of p,p'-DDT (ppm) in milk after delivery. Open circle indicates patient 1; solid circle, patient 2; open square, patient 3; solid square, patient 4; and x, patient 5.



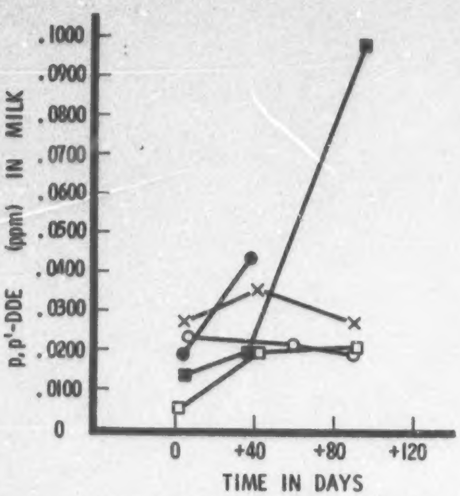


Fig 8.—Individual concentration of p,p'-DDE (ppm) in milk after delivery. Open circle indicates patient 1; solid circle, patient 2; open square, patient 3; solid square, patient 4; and x, patient 5.

DDT, increased in plasma during the lactation period studied. These findings suggest but do not prove that delivery leads to lower plasma levels of these compounds.

A variety of physiological changes that occur in pregnancy could affect the plasma level of the chlorinated hydrocarbon insecticides. During this period serum albumin values decrease (25%), the total serum lipid values increase (46%), progesterone and estrogen production is increased, water is retained to a total volume of about 7 liters in normal pregnancy, and the blood volume increases in late pregnancy.[11] Any one or a combination of these factors may influence the level of chlorinated hydrocarbon insecticides in plasma. Furthermore, the subjects received a variety of drugs (Table 1) during and after delivery which might have stimulated the activity of oxidative enzymes in the liver microsomes.[12,13]

The levels of the studied insecticides in milk showed a great deal of individual variation and were within the range of those reported by others.[1-3,5,6,8] Conversely, Damaskin[4] reported much higher values.

The number of subjects was small, and the apparent increase of *p,p'*-DDE in the milk in the later part of the lactation period must be considered with great reservation since it was not statistically significant. A correlation between the milk and plasma levels of the studied compounds could not be established.

Mrs. Estelle Gray assisted with the statistical analysis, and Mrs. Catherine L. Thrasher and Miss Helen Mann assisted with the insecticide determinations.

Generic and Trade Names of Drugs

Meperidine—*Demerol.*
Propoxyphene hydrochloride—*Darvon.*
Secobarbital—*Seconal.*
Promethazine—*Phenergan.*
Pentobarbital—*Nembutal.*
Propiomazine hydrochloride—*Largon Hydrochloride.*
Chlorpromazine—*Thorazine.*
Levallorphan—*Lorfan.*

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Trans-Nonachlor Residues in Human Adipose Tissue

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trans-Nonachlor, chemically known as 1,2,3,4,5,6,7,8,8-nonachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane, is a component of technical chlordane and technical heptachlor. According to Interpretation No. 23 of the Federal Insecticide, Fungicide and Rodenticide Act, as amended, technical chlordane is defined as containing 60% octachloro-4,7-methanotetrahydroindane and 40% related compounds. *trans*-Nonachlor is one of several chemicals found as a part of these related compounds and has been detected in technical heptachlor (Cochrane et al. 1970). Chlordane has been registered with the federal government since 1948 as an economic poison. It has been widely used as an insecticide and to a lesser extent, as a herbicide. Since its insecticidal efficacy includes pests of turf and lawns as well as household pests, its usage tends to bring this chemical in close proximity to humans.

The objective of this article is to report the discovery of *trans*-nonachlor in human adipose tissue and to provide a preliminary assessment of its geographic distribution in the United States. Although this report ascribes no clinical or symptomatic repercussions to these residues in humans, the finding of *trans*-nonachlor is indicative of exposure to chlordane and heptachlor. Other chemicals which are representative of chlordane and heptachlor exposure have also been reported in human tissues. Oxychlordane, a mammalian metabolite of several chlordane constituents, has previously been reported (Biros and Enos 1973). Heptachlor epoxide, derived from heptachlor or possibly in part from the heptachlor in technical chlordane, has also been reported in a national survey of human adipose tissue (Kutz 1974). *trans*-Nonachlor has also been reported in some environmental samples (Law and Goerlitz 1974, Lichtenstein 1971).

trans-Nonachlor was first detected as an unidentified component of adipose tissue extracts at the Pesticide Laboratory, Michigan Department of Public Health, which performs chemical analyses under contract for the National Human Monitoring Program (Kazen et al. 1974). These extracts were sent to the Environmental Toxicology Division, Research Triangle Park, N.C., for chemical characterization. When it was determined

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that the component was trans-nonachlor (Sovocool and Lewis 1975), a special study was organized under the National Human Monitoring Program to provide some preliminary data on the chemical epidemiology of this residue.

The National Human Monitoring Program functions to determine, on a national scale, the incidence and level of exposure to pesticides experienced by the general population and to identify changes and trends in these parameters when they occur. Further program information is detailed by Yobs (1971).

MATERIALS AND METHODS

Human adipose tissue was collected at various geographic locations throughout the conterminous United States. These tissues were extracted and analyzed for organochlorine pesticides using gas liquid chromatography with electron capture detection (GLC-EC). The presence of trans-nonachlor was confirmed, where possible, with gas liquid chromatography combined with mass spectrometry (GLC-MS). Residue levels of selected extracts were further estimated by multiple ion detection mass spectrometry (MID-MS).

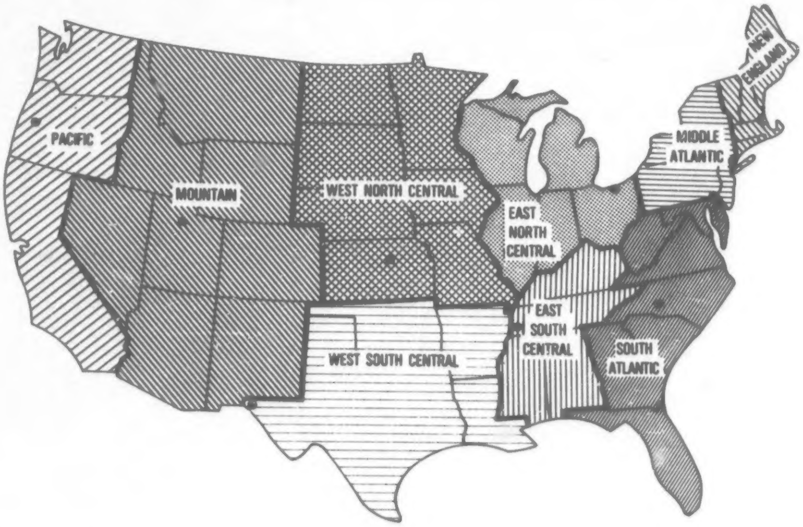

FIGURE 1

MAP OF THE UNITED STATES SHOWING THE LOCATION OF THE COLLECTION SITES IN THE 9 CENSUS DIVISONS

Five of the tissue specimens submitted from each of these sites during FY 1974 were extracted; these extracts were pooled to make a composite sample for each collecting site in each census division. The demographic (age, sex and race) description of the patients sampled to make up each composite are summarized in Table 1.

TABLE 1
Demographic Description of Patients Sampled for *trans*-Nonachlor Analysis

Census Division	Geographic Location Sampled	No. of Patients Composited	Caucasian Female	Caucasian Male	Negro Female	Negro Male	Age Range	Samples Collected During
New England	Massachusetts	5	1	3	1	0	23-68	Postmortem
Middle Atlantic	New York	5	0	5	0	0	56-82	Postmortem
East North Central	Ohio	5	2	3	0	0	18-80	Postmortem
West South Central	Texas	5	4	1	0	0	10-65	Postmortem
South Atlantic	North Carolina	5	3	1	1	0	17-50	Postmortem
East South Central	Tennessee	5	4	1	0	0	25-77	Postmortem
West North Central	Kansas	5	3	2	0	0	41-67	Surgery
Mountain	Utah	5	2	2	1	0	22-73	Postmortem
Pacific	Oregon	5	4	1	0	0	29-43	Surgery

Sample Collection

Samples of human adipose tissue were obtained through cooperating medical pathologists and medical examiners at hospitals in cities selected according to a proportionate, stratified-random design. The conterminous 48 states were divided into 9 census divisions, according to the 1970 census of the United States. A city within each census division was selected from those already participating in the National Human Monitoring Program as the collection site for this special project (Figure 1).

Chemical Analysis

All tissues were extracted and cleaned-up according to a modified Mills-Olney-Gaither procedure (Thompson 1972) by laboratories under contract to the National Human Monitoring Program. Concentrated extracts, corresponding to the 6% diethyl ether in petroleum ether fraction from the florisil clean-up column were sent to the Environmental Toxicology Division for GLC-MS analysis.

Gas Chromatograph: Hewlett Packard 5700A with 1.8mm by 2mm i.d. X 4mm o.d. glass column packed with 1.5% OV-17, 1.95% OV-210 on 80-100 mesh Gas Chrom Q. The GC inlet temperature was 200°C. Helium flow rate was 42 ml/min. Temperature programmed runs were made from 80°C (2 min.) to 210°C at 8°C/min. Multiple ion detection runs were made isothermally at 190°C. The transfer line to the mass spectrometer was maintained at 210°C and the silicone membrane separator was kept at 180°C.

Mass Spectrometer: A Hewlett Packard 5930A quadrupole focusing mass spectrometer operated at 70eV electron energy and 400µ amps filament emission with target current of 310µ amps. MID runs used 100µ amps and high electron multiplier voltages. The ion source temperature was maintained at 180°C and the mass filter temperature at 110°C. Ions were detected using a Bendix continuous dynode electron multiplier with voltages varied between 1.5 and 2.5 Kv, as required for sufficient signal strength. Data were collected, stored and plotted using a Hewlett Packard 5932A Data System. Multiple ion detection was controlled by a suitable program provided by the manufacturer, and by the data system. The ions monitored were 405, 407, 409, 411 m/e. Data output also involved the use of a light beam oscillograph, as well as plotted mass spectra and reconstructed total ion chromatograms from the data system.

Using the periodic scanning mode of operation, with the Hewlett Packard 5932A Data System, mass spectra were generally scanned from 100 to 550 m/e at 160 m/e per sec., with scan cycle times of about 5.5 sec.

A standard sample of trans-nonachlor was secured from Velsicol Chemical Corporation, Chicago, Illinois. The melting point and mass spectrum of the standard were found to be in agreement with the literature (Damico et al. 1968 and Cochrane et al. 1970).

The technique of multiple ion detection (MID) was employed to estimate the trans-nonachlor levels in the samples from Massachusetts and Texas. Quantification by GC/MS was difficult to achieve, since no completely reliable method has yet been developed for trans-nonachlor.

Confirmation was based on the presence of the large M^+-Cl ion at 405 m/e (8Cl), and where possible, the much weaker molecular ion (M^+) at 440 m/e (9Cl). The weaker retro-Diels-Alder fragment ions were occasionally hidden under ions resulting from components in the adipose tissue. Gas liquid chromatographic retention times of standard trans-nonachlor were checked for all positive samples and were found to be identical to mass spectrum and GLC retention time of the compound identified in the human adipose tissue samples.

RESULTS AND DISCUSSION

The results of the analysis of human adipose tissue for trans-nonachlor are presented in Table 2. Of the nine composite samples analyzed, eight showed confirmable levels of trans-nonachlor. Estimates using the minimum detection limits indicated that levels in all positive samples were greater than 0.01 ppm. The MID estimates of residue levels in two samples are shown in parentheses.

TABLE 2

RESULTS OF ANALYSIS FOR TRANS-NONACHLOR IN HUMAN ADIPOSE COMPOSITE EXTRACTS

Census Division	GLC-Mass Spectrometry Confirmation
New England	Confirmed (0.04 ppm)[1]
Middle Atlantic	Confirmed
East North Central	Not Confirmed
West South Central	Confirmed (0.06 ppm)[1]
South Atlantic	Confirmed
East South Central	Confirmed
West North Central	Confirmed
Mountain	Confirmed at a lower level than others
Pacific	Confirmed

[1] Concentration estimated by multiple ion detection using the intensity of 409 m/e ion.

Positive samples were found both in widely diverse geographic areas as well as in widely diverse demographic distributions. The positive composites included samples of males and females, caucasians and negroes, different age groups and samples collected at both surgery and postmortem. Postmortem specimens were collected from hospital cases as well as from coroner/medical examiner sources. Therefore, residues were found in tissues received from patients having pathological conditions and from those killed by traumatic injuries.

One composite sample from Ohio did not contain confirmable quantities of trans-nonachlor. Although the composite sample from Utah contained confirmable quantities of trans-nonachlor, the level was lower than in the other positive samples. The exact explanation for the apparent absence of trans-nonachlor from the Ohio sample and for the low level in the Utah sample cannot be determined from this study. It is, however, important to note that this is a preliminary report containing data derived from a small subsample of the national human monitoring survey.

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